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Synthesis of Fluoroflavones as Potential Neuroprotective Agents

Maali Alshammari
University of Mississippi

SYNTHESIS OF FLUOROFLAVONES AS POTENTIAL NEUROPROTECTIVE AGENTS

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Maali D. Alshammari
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ABSTRACT

Flavones are polyphenol natural products that are known to have neuroprotective effects against many diseases caused by the formation of reactive species. Flavonoids are subgroup of flavones and they are believed to function as antioxidants. Specifically, they target free radicals and eliminate its harmful effect on the cell.

Fluoroflavones were designed to improve the potency of the antioxidant activity and potentially be used as neuroprotective agents. A monofluorinated and trifluoromethylated flavone were synthesized by using commercially available fluorination reagents, *N*-fluorobenzenesulfonimide; and Togni's or Umemoto's respectively. After the synthesis of the desired flavones and their monofluorinated and trifluoromethylated derivatives, biological testing was conducted. First, antioxidant activity was evaluated by using DPPH antioxidant assay and displayed that the fluorinated flavones are more potent compared to their non-fluorinated derivatives. Second, ^{19}F NMR experiments were conducted to investigate the effect of fluorination on the antioxidant activity of flavones

DEDICATION

For my family, friends and my country.

LIST OF ABBREVIATIONS OR SYMBOLS

°C	Celcius
CoQ10	Coenzyme Q10
DPPH	2,2-diphenyl-1-picrylhydrazyl
EC_{50}	Half maximal effective concentration
FDA	Food and drug administration
GSH	Glutathione
Hex	Hexanes
h	Hour
IC_{50}	Half maximal inhibitory concentration
LPH	Lactase- phlorizin hydrolase
MBP	Metal-binding proteins
MEL	Melatonin
NFSI	N-fluorobenzenesulfonimide
NMR	Nuclear magnetic resonance
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
rt	Room temperature
SAR	Structure–activity relationship
THF	Tetrahydrofuran
TLC	Thin layer chromatography
UA	Uric acid
UGT	UDP-glucuronosyltransferase
UV	Ultraviolet

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CHAPTER 1

INTRODUCTION

1.1 Flavonoid:

Polyphenols are secondary metabolites found in many plants. All characterized by the presence of phenol rings, and they can be subdivided to two main classes depending on their structure; flavonoid and non-flavonoid. The non-flavonoid includes: stilbenes, lignans and phenolic acids, which can be further divided into two main types, benzoic acid and cinnamic acid, while flavonoids are subdivided into six main types which will be discussed later. [1]

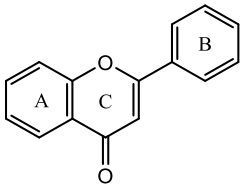


Figure 1.1: Basic structure of flavonoids.

1.1.1 Flavones: A Polyphenol Natural Product:

Flavones are a subgroup of flavonoids, which are synthesized in plants from three building blocks phenylalanine, tyrosine, and malonate [2]. The general structure of flavonoids consists of three six-membered rings, two aromatic carbon rings, benzopyran (A and C rings) and benzene (B ring), that vary in the degree of oxidation and substitutions (Figure 1). Flavonoids can be divided into six subgroups based on the degree of the oxidation of ring C. The main subgroups are (1) flavonols, (2) flavones, (3) isoflavones, (4) flavanones, (5) flavanols, and (6) anthocyanidins as shown in (Figure 2). [3]

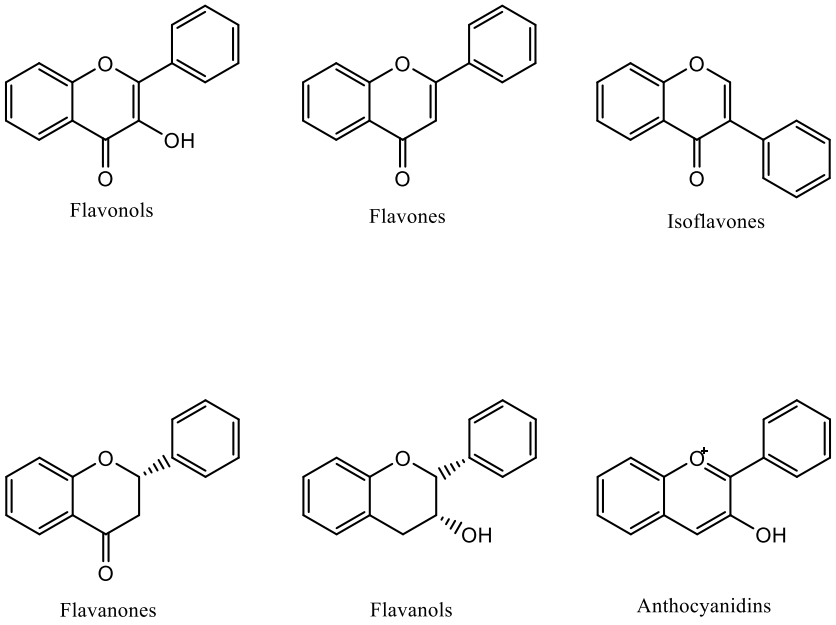


Figure 1.2: Classes of flavonoids.

Flavonoids are generally found in vegetables and fruits like apples, cauliflower, and carrots, and also in beverages like tea, coffee and red wine. Flavonoids give fruits, flowers and even the leaves different shades of color which will help the plant to attract pollinating insects, and in contrast, flavonoids can have a protective response to harmful insects. Because of their ability to absorb UV radiation, flavonoids can absorb the harmful sun radiation and function as catalysts in many photosynthesis processes. [4]

The total dietary intake of flavonoids as antioxidants range between 50 and 1000 mg/day, which is very high when compared to other natural antioxidants like vitamin C and vitamin E, that are around 10 and 100 higher than flavonoids daily dose, respectively. [5] Flavonoids can be absorbed in many forms, either with the glycosides or without it, or even as phenolic acids. All of the three forms have antioxidant activity in different degrees.

1.1.2 Metabolism of Flavones

In general, flavonoids undergo extensive metabolism by methylation, glucuronidation or sulfation; due to the several hydroxyl groups found in their structures. This rapid metabolism affects its absorption after oral administration. Fortunately, one of these enzymatic pathways (O-methylation) will increase the stability and distribution of flavonoids and hence increase their bioavailability [5]. The majority of flavones in plants are found conjugated as 7-O-glycosides, and the metabolic transformations will effect both the glycone moiety and the aglycone flavone. The major metabolic conversions are: 1) conversion of flavone 7-O-glucoside to flavone aglycone by lactase-phlorizin hydrolase (LPH) in small intestine, 2) conversion of flavone aglycone to flavone glucuronide by UDP-glucuronosyltransferase (UGT) in both liver and small intestine, 3) conversion of aglycone flavone to the flavone sulfate by sulfotransferase in the liver, and 4) conversion of flavone 7-O-glucoside to flavone aglycone and 3-(4-hydroxyphenyl) propionic acid by normal flora of large intestine (Figure 3). [6]

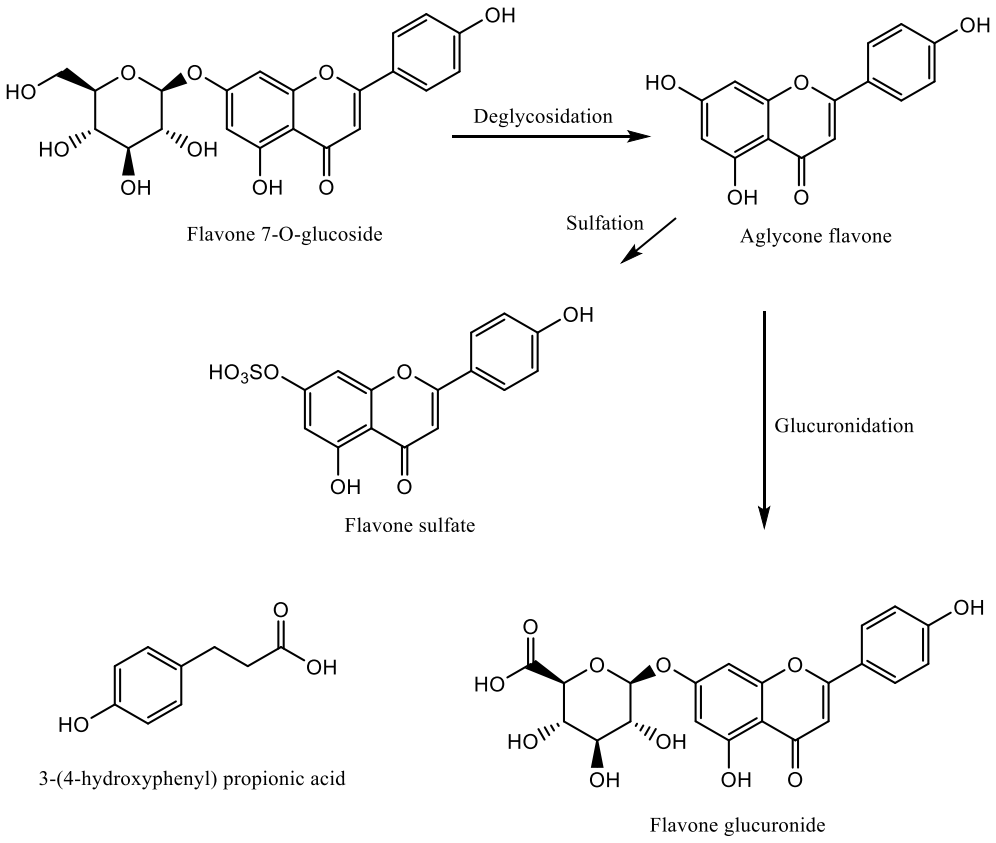


Figure 1.3: Metabolism of flavones

1.1.3 Flavones as antioxidants:

Flavonoids display three key structural features in order to function as radical scavenger; ring B must have a catechol group, ring C must have a 2,3-double bond in conjugation with a 4-oxo group, ring A must have two hydroxy groups at 3- and 5. All the previous points are responsible for electron delocalization that is necessary to stabilize the free radicals [7] (Figure 1.4). It was reported that flavonoids function as antioxidants by a radical scavenging mechanism. Although this is true in vitro, a different mechanism is has been proposed in vivo. Ursini suggested that flavonoids induce endogenous antioxidant activity by a signaling pathway rather that radical scavenging. [8] This pathway involves oxidative activation of the NF-E2-related factor. It has been reported that radical scavenging is not as effective in vivo when it's compared with other stronger endogenous antioxidants. [8]

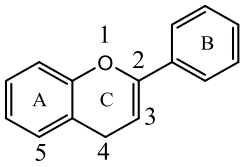


Figure 1.4: Structure of flavone.

1.2 Oxidation:

In chemistry, oxidation is defined as the removal of electrons from one reactant to another. Oxidation is always linked with reduction reactions and are usually referred as redox reactions. In biology, the same concept is applied. [9]

1.2.1 Oxidants:

Reactive species belong to two types of reactive molecules: reactive oxygen species (ROS) and reactive nitrogen species (RNS). The term ROS include both free radical and non-free radical species like hydrogen peroxide, molecular oxygen, and ozone. [10] ROS is a normal byproduct in many aerobic metabolisms and is regulated by NADPH. [11] It has an essential function in cellular signaling and communications. Damage to cellular tissue may occur when the intracellular level of ROS is elevated, which is known by oxidative stress.

1.2.2 Antioxidants:

An antioxidant is a nucleophilic reductant that prevents oxidation of an another molecule, either by reducing the free radicals or preventing their formation. [9] It can be endogenous and exogenous. The endogenous include two main categories which are both complementary to enzymatic and non-enzymatic defenses. The enzymatic defenses are enzymes that prevent the formation of free radicals by metabolizing reactive oxygen species like hydrogen peroxide, superoxide, oxygen singlet, and lipid peroxides. Examples of antioxidant enzymes include Cu/Zn-superoxide dismutase, catalase, Se-glutathione peroxidase and glutathione reductase. [2] The non-enzymatic defenses can inactivate free radicals immediately, and they are further subdivided into intracellular and extracellular processes. The most common ones are the metal-binding proteins (MBPs), coenzyme Q10 (CoQ10), glutathione (GSH), uric acid (UA) and melatonin (MEL).

The question is, how these different types of antioxidant complement each other? The answer is summarized in the first, second, and third line of defenses against ROS. [12] The first line is the most powerful one and it includes both enzymatic and non-enzymatic defenses. Each one of these antioxidant functions in a distinctive manner. The Cu/Zn-superoxide dismutase is a metalloenzyme that catalyzes the disproportionation (simultaneously oxidization and reduction) of superoxide free radical (O2•–) to form hydrogen peroxide and molecular oxygen (Table 1.1). Another enzymatic defense is catalase (CAT) which is a heme-containing enzyme that can covert hydrogen peroxide to molecular oxygen and water in a two-step reaction, a reduction and oxidation.[13] Furthermore, Se-glutathione peroxidase is a selenoenzyme in which hydrogen peroxide is reduced to water and glutathione (GSH) is oxidized to the glutathione disulfide (GSSG). [14]

The other part of the first line defenses is the non-enzymatic substances which are proteins that inhibit the formation of free radicals by transition metal ion binding mechanism, examples include, albumin ceruloplasmin and ferritin. Those non-enzymatic substances that have the ability to rapidly inactivate free radicals, like coenzyme Q10 (CoQ10), glutathione (GSH), uric acid (UA) and melatonin (MEL). The third line of defense is the repairing step which involve enzymatic antioxidants that will repair the damage generated by free radicals in DNAs, proteins, cell membrane and other cell organelles. [9]

Table 1.1: Main endogenous antioxidant.

Name of the enzyme	Main target	The resulted reaction
Cu/Zn-superoxide dismutase	superoxide free radical	$2\ HO_2 \rightarrow O_2 + H_2O_2$
Catalase	hydrogen peroxide	$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$
Se-glutathione peroxidase	hydrogen peroxide	$2GSH + H_2O_2 \rightarrow GS–SG + 2H_2O$

Endogenous antioxidants are known to be stronger than exogenous ones, but the balance between ROS and antioxidants is always changing. In other words, oxidative stress is happening, whether the cause was from internal factors like inflammation or cancer or external factors like environmental pollutants such as smoking, radiations or even metals. Also, normal factors like aging can affect oxidation state. The need for exogenous antioxidants is to support the endogenous antioxidants and prevent any possible damage generated by the free radicals is clear. Exogenous antioxidants are substances that can be found in diet or from certain supplements. The most common exogenous antioxidant are vitamins like vitamin E and vitamin C, and polyphenols, like flavonols, flavones, and anthocyanidins. Most the dietary antioxidant can be found in fruits, vegetables and fruit-based beverages line wine and tea.

1.3. Fluorine in Medicinal Chemistry

Introducing a fluorine atom into a biologically active molecule is known to produce a significant impact. It is believed that 25% of drugs in the pharmaceutical market contain at least one fluorine atom. [15] Having high electronegativity and a small molecular size makes fluorine atom valuable in drug design. [16] Hydrogen and fluorine have similar molecular size, which makes fluorine a good alternative for the strict requirements at enzyme receptor sites, [17] even though their Van Der Waals radii are not similar (F = 1.47 Å, H = 1.20 Å). [18] In addition, fluorine often increases lipophilicity and therefore improves the pharmacokinetics properties like absorption and distribution [17] and enhances binding affinity. [16] 5-Fluorouracil (Efudex®) is one of the oldest fluorinated drugs (Figure 1.5). This antineoplastic agent was synthesized by Heidelberger in 1957 and function by interfering with the synthesis of thymidylate. [15]

Another well-known example of fluorine containing drugs is the antidepressant fluoxetine which was approved by the Food and Drug Administration (FDA) in 1987. Fluoxetine (Prozac®) contains a trifluoromethyl group in the para-position of the phenol ring. The structure–activity relationship indicates that the addition of trifluoromethyl increases the selectivity for the serotonin receptor by 6-fold when compared to the non-fluorinated parent compound. [15]

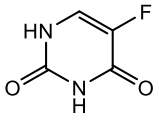


Figure 1.5: Structure of 5-fluorouracil

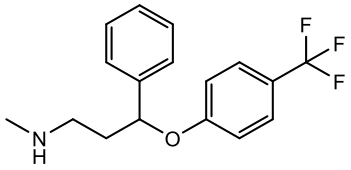
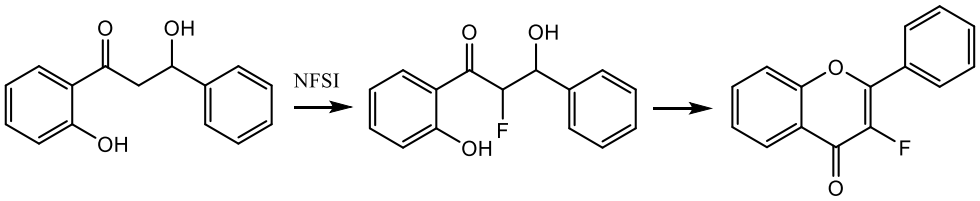


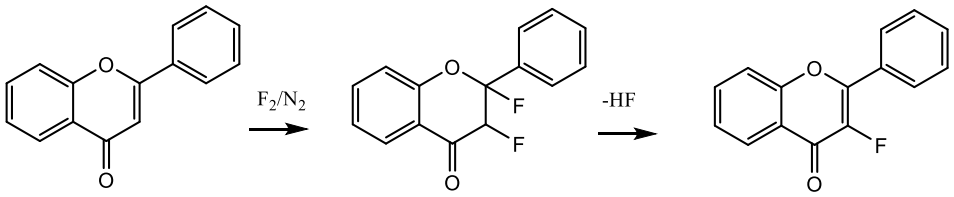
Figure 1.6: Structure of fluoxetine

1.4. Fluorinated Flavonoids

Flavones are fluorinated to increase their potency by preventing the extensive metabolism, like methylation, glucuronidation or sulfation. Many fluorinated flavonoids have been the synthesized by using a variety of methods. A fluoroflavone can be accessed by using acetophenone derivatives as starting materials followed by electrophilic or radical fluorination. [16] The other method involves the use of elemental fluorine on a flavone followed by dehydrofluorinaion to the monofluoroflavone by adsorbing the intermediate on a silica gel (scheme 1.2).



Scheme 1.1: Fluorination of flavone by using fluorinating reagents.



Scheme 1.2: Fluorination of flavone by using elemental fluorine. [15]

1.4.1 Monofluorinated Flavone

Many fluorinated flavones has been synthesized and tested in the literature. One example is the work of Vanotti in which the 3-fluorotetrahydroxy flavone was synthesized and tested against cancer as telomerase inhibitors. The fluorinated flavone displayed a 13-fold reduction in IC_{50} for inhibition of telomerase activity compared to the non-fluorinated molecule. The IC_{50} of 3-fluorotetrahydroxy flavone was 0.6 μM while for 3',4',7,8-tetrahydroxyflavone it 7.8 μM. [20]

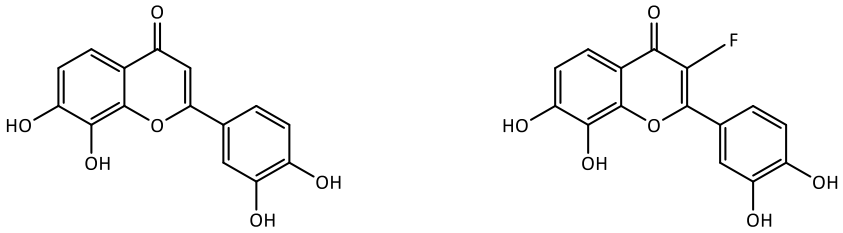
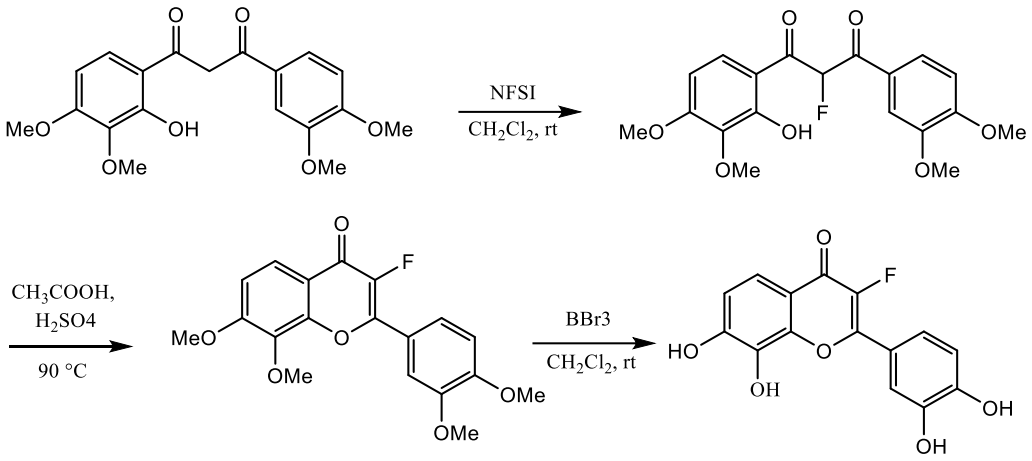


Figure 1.7: Structure of 3',4',7,8-tetrahydroxyflavone and 3',4',7,8-tetrahydroxyfluoroflavone

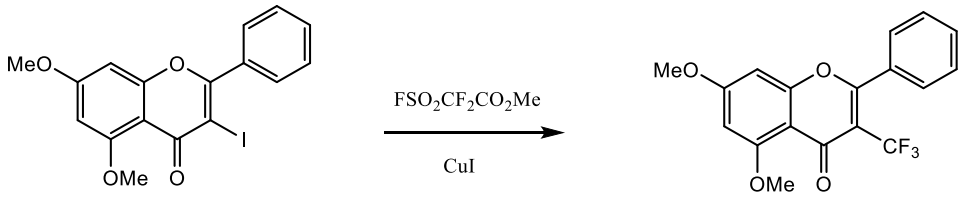
The 3-fluorotetrahydroxy flavone was synthesized by reacting N-fluorobenzenesulfonimide with the diketone intermediate in anhydrous dichloromethane for seven days to afford the product in 28% yield. The mono-fluorinated intermediate was then cyclized by refluxing in acidic conditions to give the product in 82% yield. Compound 4 was demethylated by using boron tribromide in dichloromethane in 71% yield. (scheme 1.3)



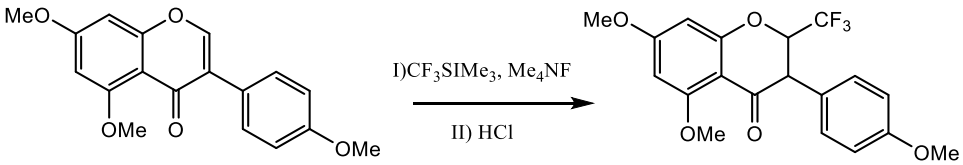
Scheme 1.3: Synthesis of monofluorinated flavone

1.4.2 Trifluoromethylated Flavone

There is only one reported trifluoromethylated flavone derivative in the literature by Qing and co-workers in 2005, in which they synthesized the trifluoromethylated flavone by trifluoromethylation of 3-iodoflavonoid derivatives with methyl 2,2-difluoro-2-(fluorosulfonyl) acetate and copper iodide as catalyst (Scheme 1.4). [21] Qing and co-workers also trifluoromethylate the flavone using trimethyl (trifluoromethyl) silane, also known as Ruppert's reagent tetramethylammonium fluoride as catalyst. The intermediate was then treated with hydrochloric acid (Scheme 1.5). [21]



Scheme 1.4: Synthesis of trifluoromethylated flavone



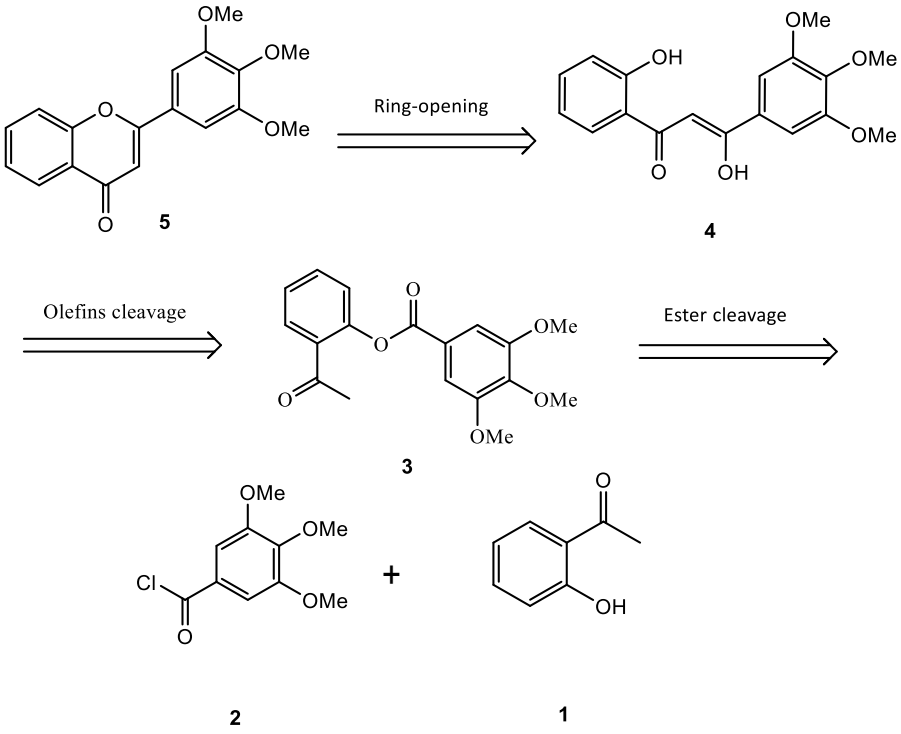
Scheme 1.5: trifluoromethylation the flavone

CHAPTER 2

SYNTHESIS OF FLAVONOIDS AND FLUORINATED FLAVONOIDS

2.1 Design strategy:

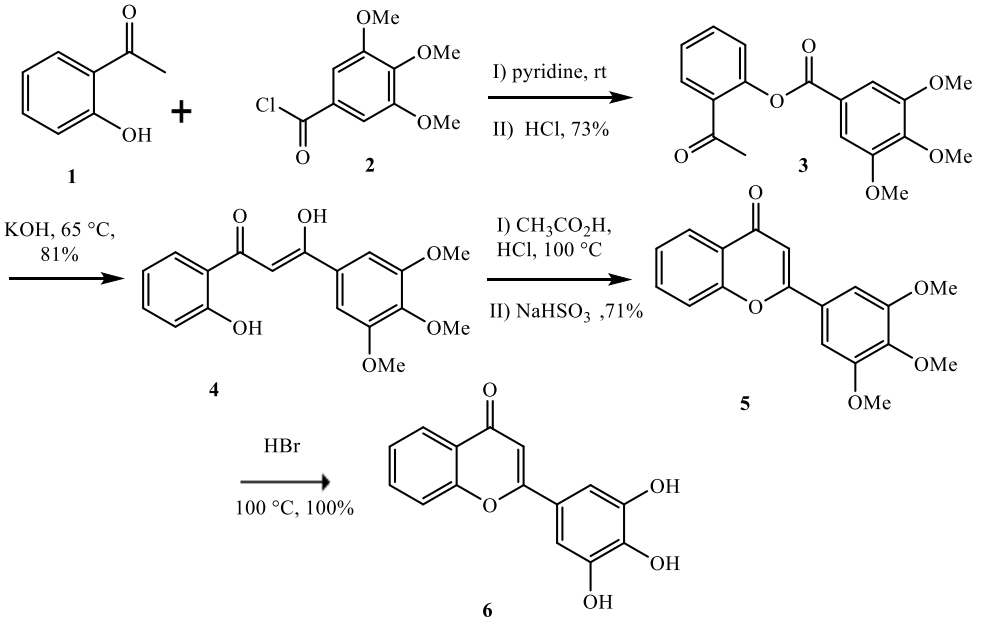
The retrosynthesis of flavone is carried out by an intermolecular cyclization of compound **4**, followed by intermolecular rearrangement of compound **3**. This arrangement is known as Baker-Venkataraman reaction which involves a reaction of compound **3** with base to form a 1,3-diketone. The last step is a simple coupling of compound **1** with **2** under basic conditions (Scheme 2.1).



Scheme 2.1: The retrosynthesis of flavone

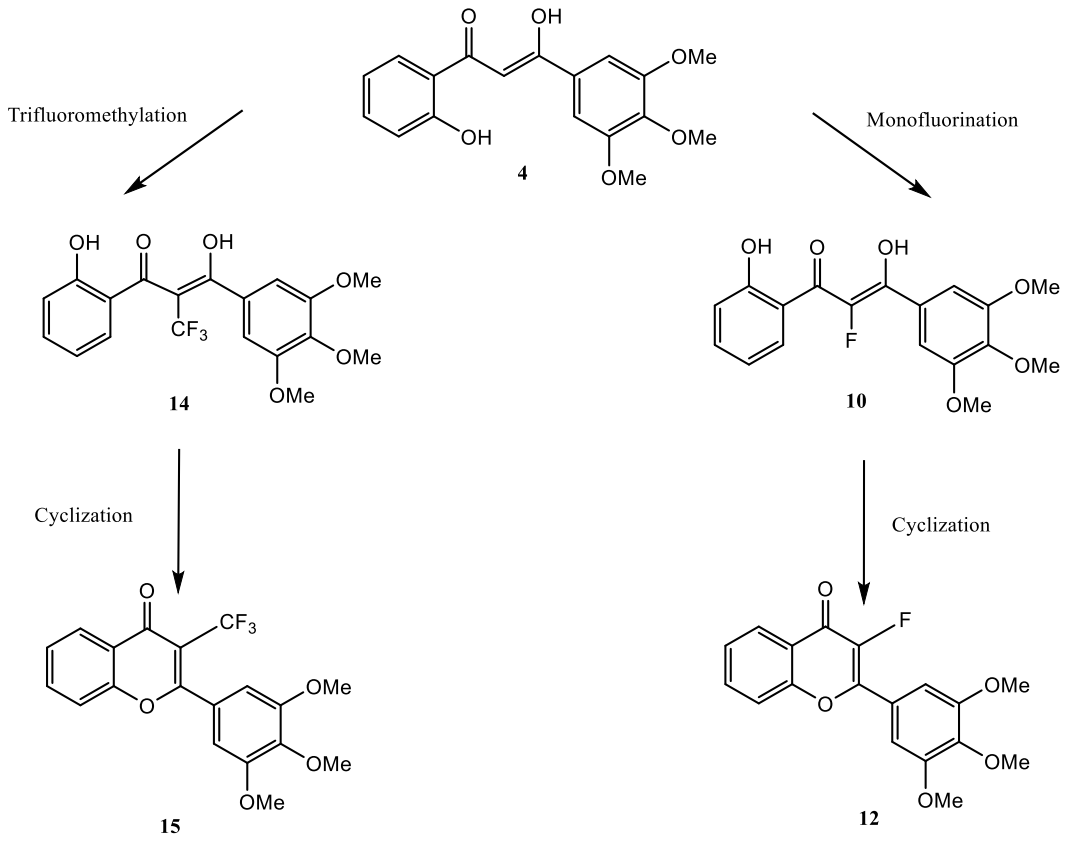
2.2 Flavonoid synthesis:

The synthesis of the desired flavone compound **4** starts with two commercially available staring materials, compound **1** (2'-hydroxyacetophenone) and compound **2** (3,4,5-trimethoxybenzoyl chloride) according to Vanotti (Scheme 2.2). [20] Coupling compound **1** with compound **2** in pyridine results in forming the ester **3** in 40% yield. Then, treating the ester **3** with potassium hydroxide forms the di-ketone **4**, by a Baker-Venkataraman rearrangement. [16] Cyclization of compound **4** by refluxing with acetic acid and hydrochloric acid and demethylation by using boron tribromide provides the target flavone **6** (Scheme 2.2).



Schemes 2.2: Route toward the desired flavone **6**

There are limited number of methods that are available for selective carbon-fluorine bond formation when compared to other carbon-halogen bond forming reactions. [18] We have two targets, which are the monofluorination and trifluoromethylation products. Both require electrophilic fluorinating reagents, and then cyclization to the targeted flavone (Scheme 2.3).



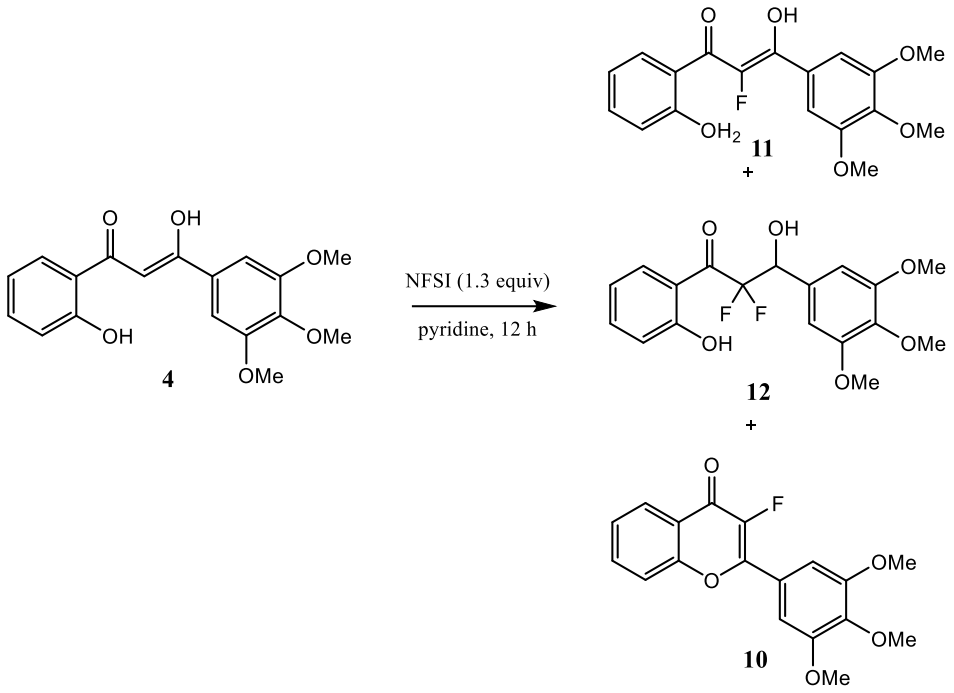
Scheme 2.3: Fluorination and trifluoromethylation approaches

2.3.Monofluorination:

N-Fluorobenzenesulfonimide (NFSI) was reported by Barnette in 1984. [18] This electrophilic fluorinating reagent is stable, easily prepared and purified. [24] The monofluorinated flavone **10** was synthesized by reacting compound **4** with NFSI for a week in dichloromethane or in a mixture of dichloromethane and acetonitrile with a yield of 28% and 37% respectively. This method uses pyridine as a solvent. We screened different temperatures to observe the selectivity and reactivity of NFSI with the diketone **4**. As the temperature increased from 0 °C to 50 °C more of the mono-fluorinated diketone **10** is observed compared to di-fluorinated diketone **11**. At 100 °C, only the cyclized fluorinated form **12** is observed which give us a more efficient and direct method to access the final product. The yield increased from 32% to 72% and the reaction time decreased from 7 days to 12 h (Scheme 2.4 and Table 2.4).

Table 2.1: Fluorination of diketone intermediate with NFSI

Entry	Temperature	^{19}F NMR ratio		
		10	11	12
1	0 °C	1	2.4	-
2	24 °C	1	2.2	-
3	50 °C	1	-	0.1
4	100 °C	-	-	1



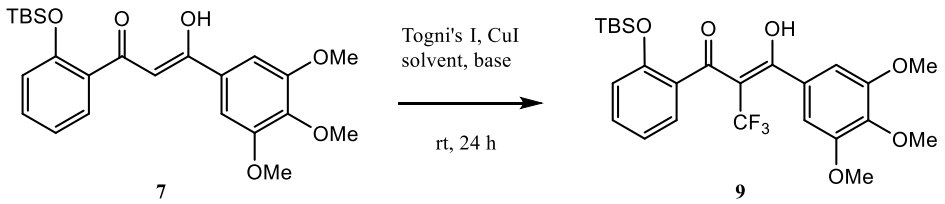
Scheme 2.4: Route toward the desired monofluorinated flavone

2.4. Trifluoromethylation

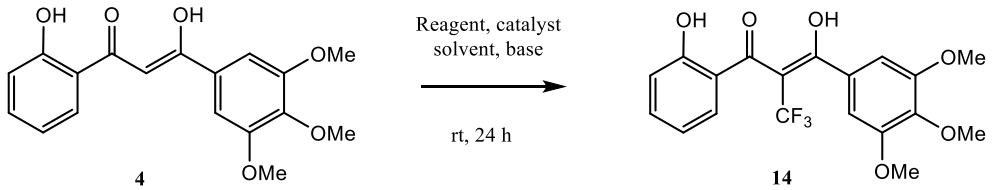
Togni I, 1-(trifluoromethyl)-1,2- benziodoxol-3(1H)-one, and Togni II, trifluoromethyl-1,3-dihydro- 3,3-dimethyl-1,2-benziodoxole, are two hypervalent iodine species that function as a source of an electrophilic trifluoromethyl group (Figure 2.1). Togni's reagent needs to be activated first by Lewis acids, Brønsted acids or even metals. Copper iodide was selected because it is one of the most widely used catalysts for activating of Togni's reagents. [25]

Figure 2.1: Structures of Togni's reagents

First, we tried to trifluoromethylate the protected β-diketone **7** with Togni I and copper iodide in THF or pyridine, with bases like LDA or BDU, or without any base. None of the reactions produced the target **9**. So we repeated the same process with the unprotected β-diketone **4**, which also did not work. Umemoto's reagent also failed to yield the desired product **9** or **14**.



Scheme 2.5: Fluorination of the protected β-diketone intermediate with Togni's reagent

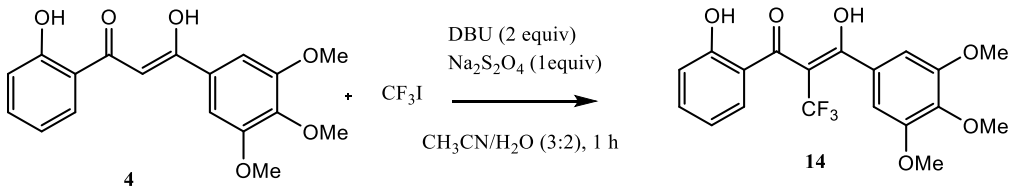


Scheme 2.6 Synthesis of fluorinated β-diketone intermediate with Togni's reagent

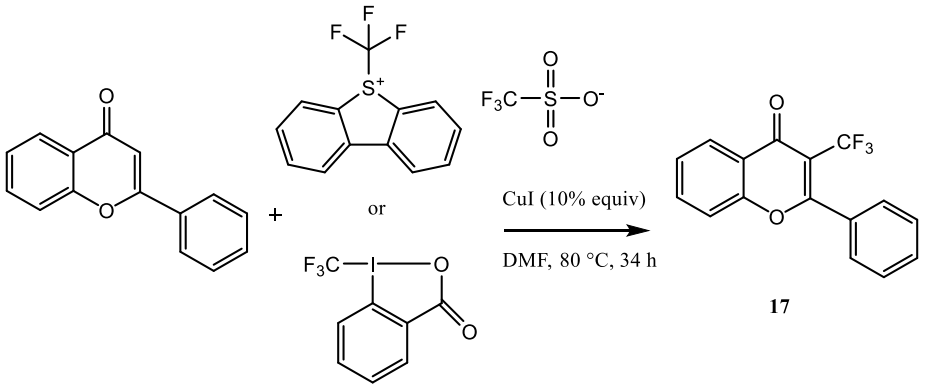
Table 2.2: Synthesis of fluorinated β-diketone intermediate

Entry	Solvent (1 mL)	Reagent [1.2]	Activating Agent	Base	Temperature (°C)	Time (h)
1	pyridine	Togni's I	CuI [0.2]	DBU	rt	24
2	pyridine	Togni's I	CuI [0.2]	—	rt	24
3	CH_3CN	Togni's II	CuI [0.2]	—	40	12
4	CH_3CN	Togni's II	CuI [0.2]	—	rt	24
5	EtOAc	Togni's II	TBAI [0.1]	—	80	24
1	CH_2Cl_2	Umemoto's	CuI [0.2]	DBU	rt	24
2	CH_2Cl_2	Umemoto's	CuI [0.2]	—	rt	24
3	pyridine	Umemoto's	CuI [0.2]	—	40	12
4	DMF	Umemoto's	TBAI [0.05]	K_2CO_3 [3]	rt	24
5	DMF	Umemoto's	TBAI [0.05]	K_2CO_3 [3]	40	24

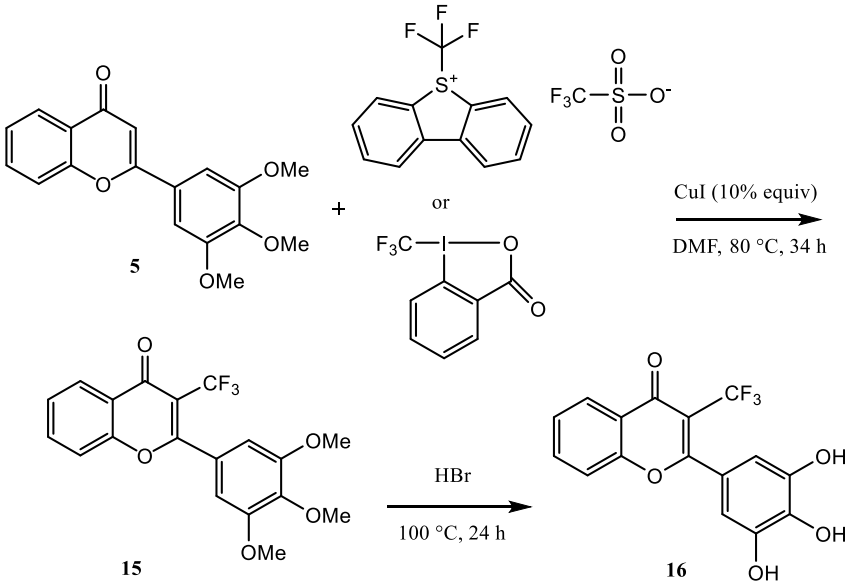
None of the previous experiment listed in (Table 2.2) led to the formation of the trifluorinated products. Another approach was taken using trifluoroiodomethane gas in a radical trifluoromethylation mechanism reagent, but again the reaction was not successful due to non-selective fluorination (Scheme 2.7). The last approach was to trifluoromethylate a flavone by using Umemoto′s reagent or Togni I and copper iodide in dimethylformamide as seen in Scheme 2.8. [26] This method resulted in the trifluoromethylation of the flavone in 20% ^{19}F NMR yield. Based on this finding, the desired methoxy flavone will be trifluoromethylated in the same fashion. The last step will involve refluxing the trifluoromethylated flavone using hydrobromic acid to afford the trihydroxyphenly flavone (Scheme 2.9).



Scheme 2.7: Radical trifluoromethylation by using trifluoroiodomethane gas



Scheme 2.8: Trifluoromethylation of flavone by using Umemoto′s and Togni′s reagent



Scheme 2.9: Trifluoromethylation of flavone by using Umemoto′s or Togni′s reagent then demethylation with hydrobromic acid

CHAPTER 3

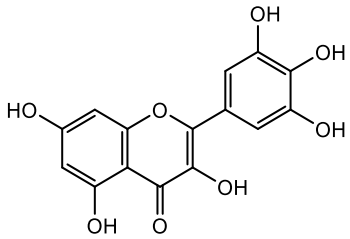
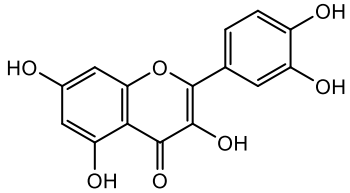
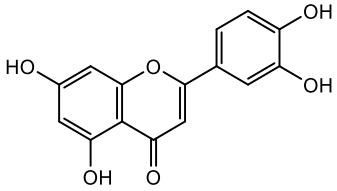
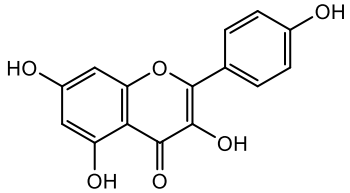
BIOLOGICAL EVOLUTION OF THE FLUORINATED FLAVONOIDS

3.1 The DPPH antioxidant assay

1,1-Diphenyl-2-picryl-hydrazyl (DPPH) is a free radical that is stable at room temperature and is a common reagent in an antioxidant assay based on electron-transfer mechanism. DPPH, produces a violet solution but when it is reduced in the presence of an antioxidant, DPPH will turn colorless. [27] The DPPH antioxidant assay is performed by using a 96-well plate and measure the scavenging activity toward the 2,2-diphenyl-1-picrylhydrazyl free radical. Many studies are conducted to measure the antioxidant activity of flavonoids using DPPH assay. [7,4]

Mira and co-workers measured the antioxidant activity of various flavonoids against DPPH radical. The flavones myricetin, quercetin, kaempferol, and luteolin were tested. Flavone and DPPH were mixed for 30 min then absorbance measured at 517 nm. The results are summarized in Table 4 and indicated how scavenging activity increases with increasing the number of the free OH group (Table 3.1). [28]

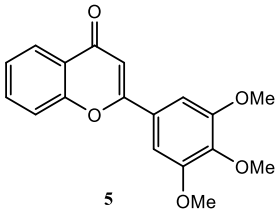
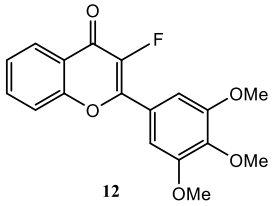
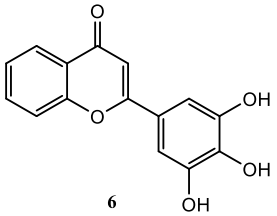
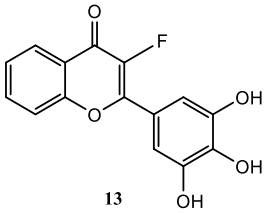
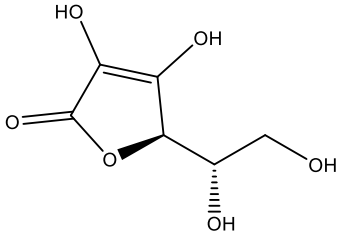
Table 3.1: DPPH scavenging activity of hydroxy-flavones

Flavones	Structure	Mmol DPPH scavenged per mmol flavonoid
Myricetin		5.7 ± 0.3
Quercetin		2.9 ± 0.1
Luteolin		2.7 ± 0.1
Kaempferol		2.2 ±0.2

3.1.1 Results

The scavenging activity of flavones 5 and 6 and their monofluorinated derivatives 12 and 13 in concentration ranging from (1.5625 – 200 μg/mL) were evaluated against 0.1 mM of DPPH by the decrease of absorbance at 517 nm. The data indicate that the monofluorinated flavones are more potent than the un-fluorinated derivatives. For example, the EC_{50} of **6** dropped from 37.14 μg/mL to 0.2359 μg/mL for the fluorinated **13**. Also, the EC_{50} of the methoxy flavone **5** dropped from 71.66 to 0.3345 μg/mL when compared with the fluorinated methoxy flavone **12** (Table 4.2) and (Figure 3.1).

Table 3.2: Flavones used in DPPH assay and their EC_{50} values.

Compound	EC_{50} ± SD (μg/mL)
 **5**	71.66 ± 0.04
 **12**	37.14 ± 0.02
 **6**	0.3345 ± 0.017
 **13**	0.2359 ± 0.035
 Vitamin C	0.1596 ±0.034

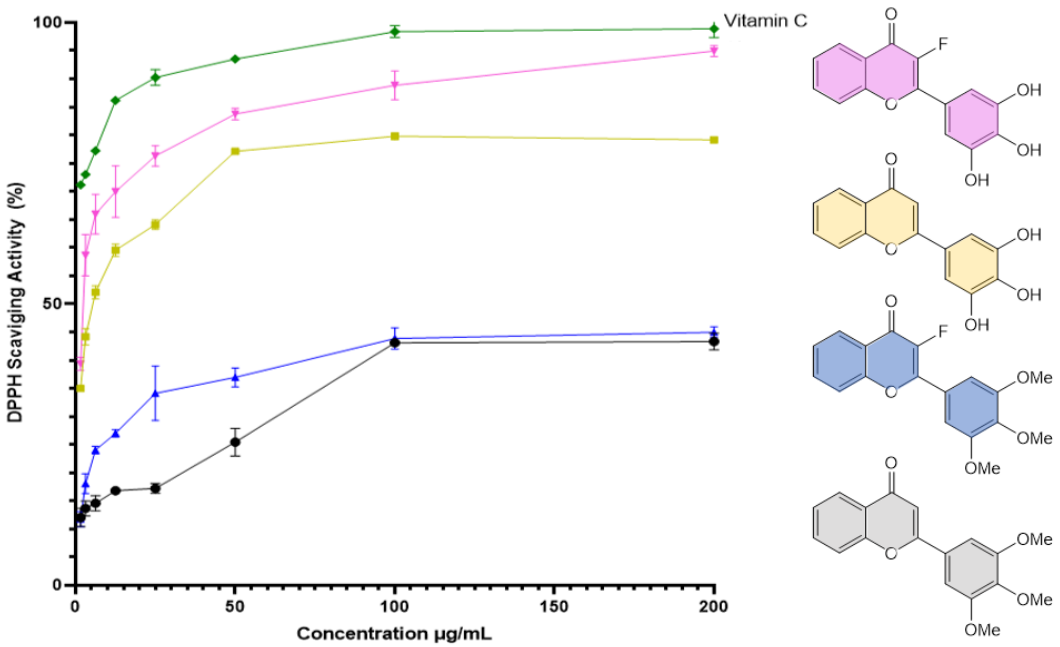


Figure 3.1: DPPH assay results

3.2 neuroprotective assay

neuroprotective assay is used to measure the cell viability in response to many substrates like growth factors, cytokines and cytotoxic reagent. In our case we will be testing the synthesized flavones and their fluorinated derivatives. This assay is based on the reduction of the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) tetrazolium compound by viable mammalian cells (neurons) to generate a colored formazan dye that is soluble in cell culture media and can be quantified by measuring the absorbance at 490-500 nm.

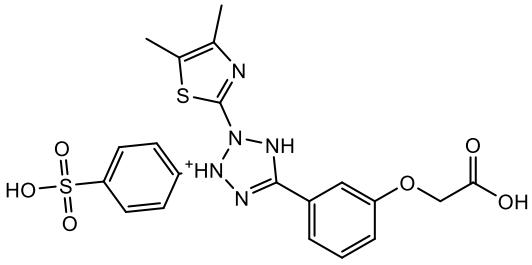


Figure 3.2: Structures of MTS

3.2.1 Results

The neuroprotective activity of flavones 5 and 6 and their monofluorinated derivatives 12 and 13 in concentration ranging from (40 – 0.31 μM) were evaluated against glutamate by measuring the absorbance of the reduced form of TMS at 490 nm. The data indicates that the un-fluorinated hydroxy flavones shows more neuroprotective activity than the monofluorinated hydroxy derivative. While the monofluorinated hydroxy derivative was more active then un-fluorinated methoxy flavones (Figure 3.3).

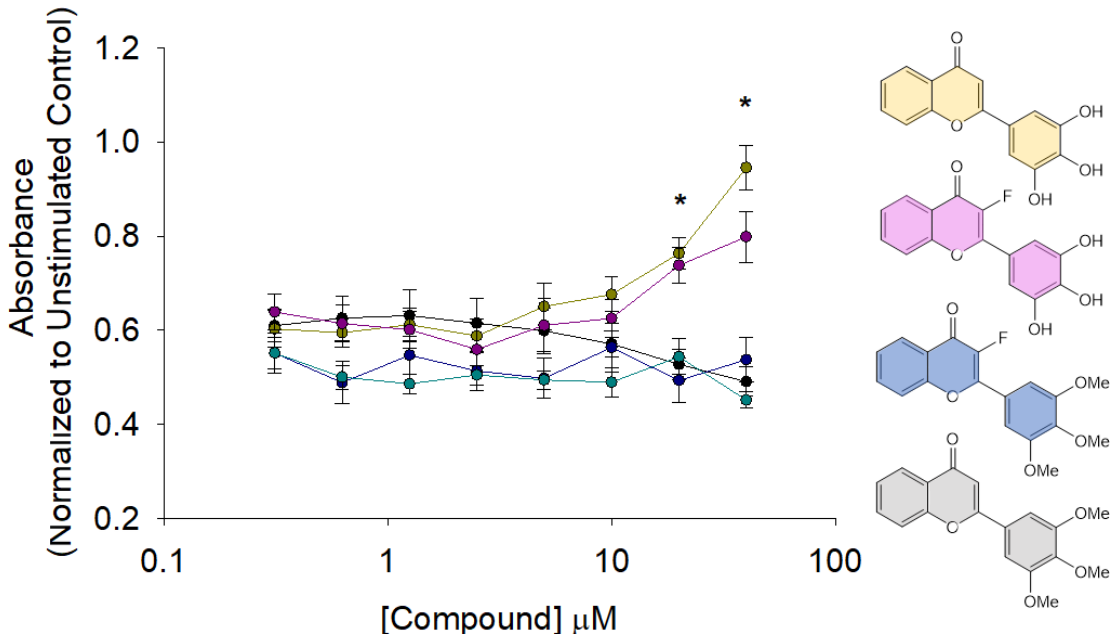


Figure 3.3: Neuroprotective assay results

CHAPTER 4

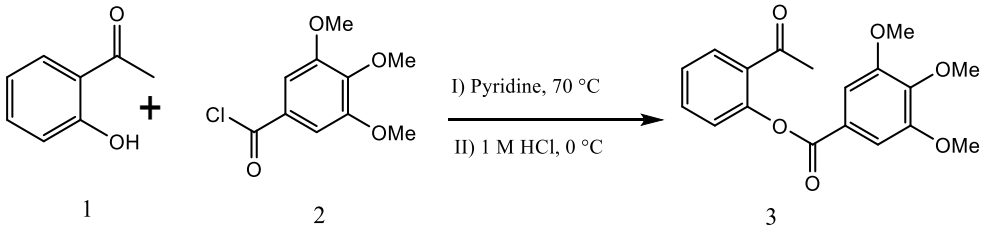
CONCLUSION

The synthesis of the desired flavones and their monofluorinated and trifluoromethylated derivative was successful. Pyridine was used in the key step to increase the yield 32% to 72% and reduce the reaction time from one week to 12 hours. The biological evaluation of the flavonoids was carried out by using two assays, DPPH antioxidant assay and MTS cell proliferation assay. DPPH antioxidant assay was preformed on the flavones and their monofluorinated flavones. The results indicate that the monofluorinated flavones are more potent than un-fluorinated flavones. Fluorinated and un-fluorinated flavones have potential neuroprotective activity. Using ^{19}F NMR to understand the impact of fluorination on the antioxidant activity of flavones is still in progress. The preliminary data are not conclusive about the effect of fluorination and therefore more NMR experiments are required.

CHAPTER 5

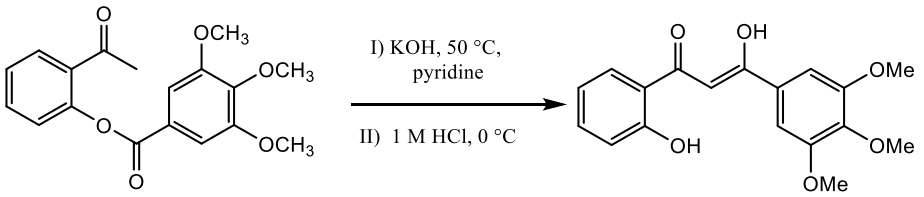
Experimental Details

5.1 Synthesis of Flavonoids



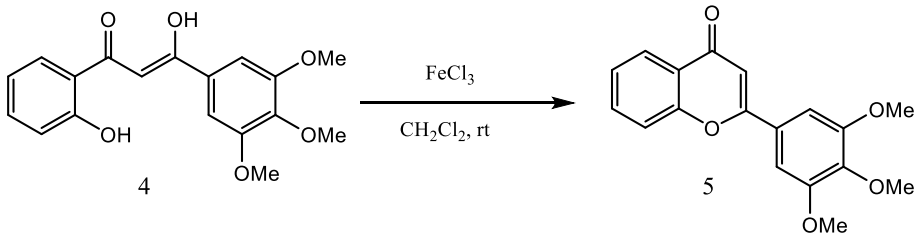
1 2 3

2-Acetylphenyl 3,4,5-trimethoxybenzoate 3. A mixture of 1-(2-hydroxyphenyl)ethan-1-one **1** (200 mg, 1.47 mmol) and 3,4,5-trimethoxybenzoyl chloride **2** (404 mg, 1.76 mmol) in pyridine (3 mL) was heated at 70 °C under air. After 12 h, the reaction mixture was cooled to 0 °C, then acidified by adding 1 M of aqueous HCl (3 mL), and the resultant mixture was extracted with EtOAc (10 mL × 3). The organics were dried over Na_2SO_4 and concentrated under reduced pressure. SiO_2 flash chromatography (7:3 hexanes/EtOAc) afforded the 2-acetylphenyl 3,4,5-trimethoxybenzoate product **3** as a white solid (357 mg) in 73% yield: ^{1}H NMR (400 MHz, CDCl3) δ 7.66 (dd, *J* = 7.8, 1.5 Hz, 1H), 7.39 (t, *J* = 8.5 Hz, 1H), 7.17 (t, *J* = 7.6 Hz, 1H), 7.06 (s, 2H), 7.03 (d, *J* = 8.1 Hz, 1H), 3.74 (s, 6H), 3.73 (s, 3H), 2.35 (s, 3H).



3 4

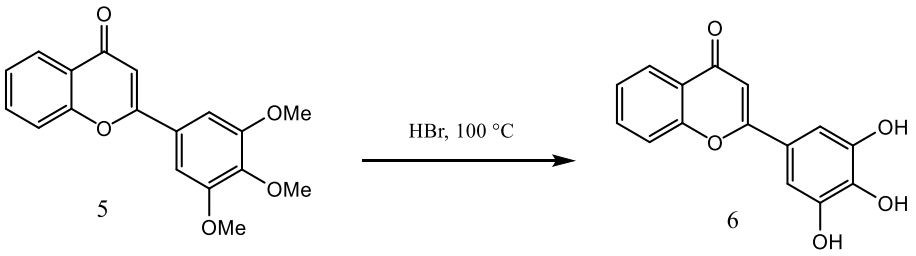
(Z)-3-hydroxy-1-(2-hydroxyphenyl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one 4. A mixture of 2-acetylphenyl 3,4,5-trimethoxybenzoate **3** (200 mg, 0.60 mmol) and potassium hydroxide (60 mg, 1.06 mmol) in pyridine (3 mL) was heated at 50 °C under air. After 12 h, the reaction mixture was cooled to 0 °C, then acidified by adding 1M of aqueous HCl (3 mL), and the resultant mixture was extracted with EtOAc (10 mL × 3). The organics were dried over Na_2SO_4 and concentrated under reduced pressure. SiO_2 flash chromatography (7:3 hexanes/EtOAc) afforded the (Z)-3-hydroxy-1-(2-hydroxyphenyl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one product **4** as a yellow solid (160 mg) in 81% yield: ^{1}H NMR (500 MHz, $CDCl_3$) δ 15.72 (s, 1H), 12.02 (s, 1H), 7.74 (d, *J* = 7.9 Hz, 1H), 7.43 (t, 1H), 7.13 (S, 2H), 6.97 (d, *J* = 8.3 Hz, 1H), 6.89 (t, *J* = 7.5 Hz, 1H), 6.71 (s, 2H), 3.93 (s, 6H), 3.91 (s, 3H).



4 5

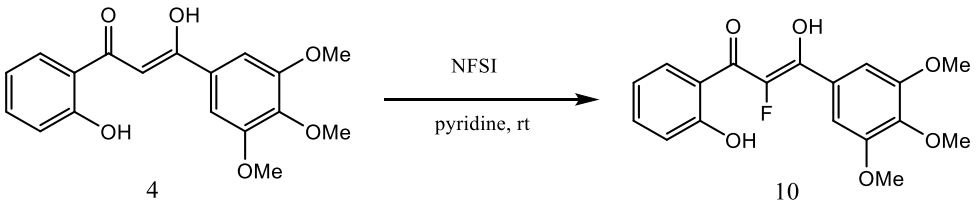
2-(3,4,5-Trimethoxyphenyl)-4H-chromen-4-one 5. A mixture of (Z)-3-hydroxy-1-(2-hydroxyphenyl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one **4** (10 mg, 0.03 mmol) and iron(III) chloride (0.47 mg , 0.0087 mmol) in dichloromethane (1 mL) was stirred at rt under air. After 24 h, the reaction mixture was extracted with dichloromethane (1 mL × 3). The organics were dried

over Na_2SO_4 and concentrated under reduced pressure to afford the 2-(3,4,5-trimethoxyphenyl)-4H-chromen-4-one **5** (8.3 mg) in 89% yield: ^{1}H NMR (500 MHz, CDCl3) δ 8.24 (d, *J* = 7.9 Hz, 1H), 7.72 (t, *J* = 7.7 Hz, 1H), 7.60 (d, *J* = 8.3 Hz, 1H), 7.44 (t, *J* = 7.3 Hz, 1H), 7.15 (s, 2H), 6.83 (s, 1H), 3.97 (s, 6H), 3.94 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.32, 163.26, 156.24, 153.64, 141.32, 133.77, 127.02, 125.72, 125.31, 123.96, 118.11, 107.40, 103.85, 77.48, 77.16, 76.84, 61.08, 56.42.



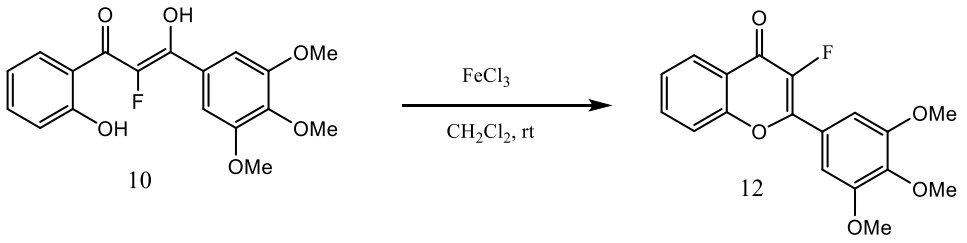
2-(3,4,5-Trihydroxyphenyl)-4H-chromen-4-one 6. A mixture of 2-(3,4,5-trimethoxyphenyl)-4H-chromen-4-one **5** (10 mg, 0.03 mmol) in hydrobromic acid (1 mL) was stirred in a sealed tube at 100 °C. After 24 h, the reaction mixture was cooled to rt and concentrated under reduced pressure to afford the 2-(3,4,5-trihydroxyphenyl)-4H-chromen-4-one **6** (8.1 mg) in quantitative yield: ^{1}H NMR (400 MHz, acetone-d_6) δ 8.10 (d, *J* = 7.5 Hz, 1H), 7.78 (t, *J* = 8.4 Hz, 1H), 7.67 (d, *J* = 8.4 Hz, 1H), 7.46 (t, *J* = 7.4 Hz, 1H), 7.14 (s, 2H), 6.60 (s, 1H), 2.80 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ 176.93, 163.67, 155.64, 146.51, 137.80, 134.22, 125.43, 124.83, 123.36, 120.79, 118.35, 105.81, 104.86, 40.15, 39.94, 39.73, 39.52, 39.31, 39.10, 38.89.

5.2 Synthesis of Monofluorinated Flavonoids

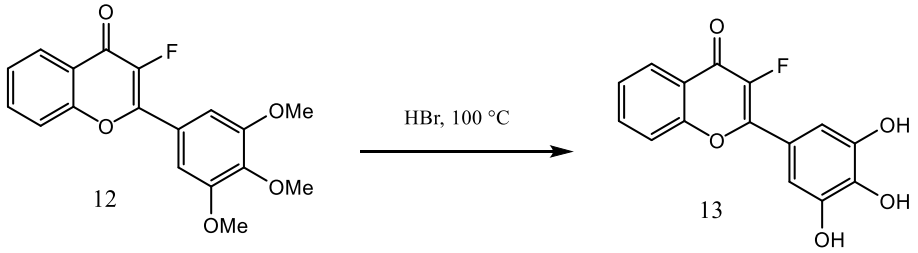


4 10

(*E*)-2-fluoro-3-hydroxy-1-(2-hydroxyphenyl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one 10. A mixture of (Z)-3-hydroxy-1-(2-hydroxyphenyl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one **4** (10 mg, 0.03 mmol), and *N*-fluorobenzenesulfonimide (11.88 mg, 0.036 mmol) in pyridine (1 mL) was stirred at rt under air. After 24 h, the reaction mixture was cooled to rt and water (2 mL) was added. The reaction mixture was cooled to 0 °C, then acidified by adding 2 M of aqueous HCl (3 mL), and the resultant mixture was extracted with Et_2O (2 mL × 5). The organics were dried over Na_2SO_4 and concentrated under reduced pressure. SiO_2 flash chromatography (7:3 hexanes/EtOAc) followed by prep. TLC by using (7:3 hexanes/EtOAc) to afforded the (*E*)-2-fluoro-3-hydroxy-1-(2-hydroxyphenyl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one **10** (5.89 mg) in 49% yield: ^{1}H NMR (500 MHz, $CDCl_3$) δ 15.72 (s, 1H), 12.02 (s, 1H), 7.74 (d, *J* = 7.9 Hz, 1H), 7.43 (t, 1H), 7.13 (S, 2H), 6.97 (d, *J* = 8.3 Hz, 1H), 6.89 (t, *J* = 7.5 Hz, 1H), 6.51 (d, *J* = 48.8 Hz, 1H), 3.93 (s, 6H), 3.91 (s, 3H);^{19}F NMR (300 MHz, $CDCl_3$) –186.17 (d, *J* = 48.9 Hz)



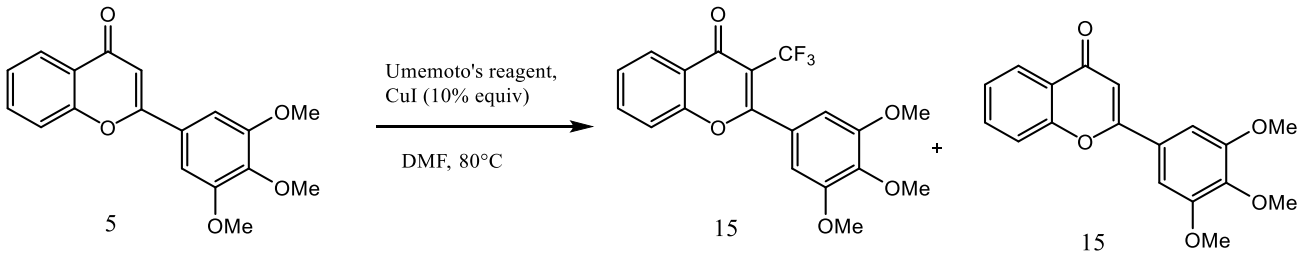
3-Fluoro-2-(3,4,5-trimethoxyphenyl)- 4H-chromen-4-one 12. A mixture of (E)-2-fluoro-3-hydroxy-1-(2-hydroxyphenyl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one **10** (10 mg, 0.0287 mmol), and iron(III) chloride (0.47 mg , 0.0087 mmol) in dichloromethane (1 mL) was stirred at rt under air, after 24 h, the reaction mixture was extracted with dichloromethane (1 mL × 3). The organics were dried over Na_2SO_4 and concentrated under reduced pressure to afforded the 3-fluoro-2-(3,4,5-trimethoxyphenyl)-4H-chromen-4-one **12** (7.9 mg) in 84% yield: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.24 (d, *J* = 7.9 Hz, 1H), 7.72 (t, *J* = 7.7 Hz, 1H), 7.60 (d, *J* = 8.3 Hz, 1H), 7.44 (t, *J* = 7.3 Hz, 1H), 7.15 (s, 2H), 3.97 (s, 6H), 3.94 (s, 3H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 170.86 (d, *J* = 16.7 Hz), 150.78 (d, *J* = 23.2 Hz), 146.09 (d, *J* = 247.8 Hz), 141.14 (d, *J* = 1.6 Hz), 125.98 (d, *J* = 3.3 Hz), 124.24 (d, *J* = 7.1 Hz), 123.86 (d, *J* = 5.1 Hz), 105.81 (d, *J* = 8.5 Hz); ^{19}F NMR (300 MHz, $CDCl_3$) –160.36



3-Fluoro-2-(3,4,5-trihydroxyphenyl)-4H-chromen-4-one 13. A mixture of 3-fluoro-2-(3,4,5-trimethoxyphenyl)-4H-chromen-4-one **12** (10 mg, 0.03 mmol) in hydrobromic acid (1 mL) was stirred in a sealed tube. After 24 h, the reaction mixture was cooled to rt and concentrated under reduced pressure to afford the 3-fluoro-2-(3,4,5-trimethoxyphenyl)-4H-

chromen-4-one **13** (8.6 mg) in quantitative yield: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.11 (d, J = 9.0 Hz, 1H), 7.90–7.80 (m, 1H), 7.74 (d, J = 8.1 Hz, 3H), 7.58–7.46 (m, 2H), 7.07 (s, 2H), 3.90 (s, 0H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.42 (d, J = 16.8 Hz), 151.19 (d, J = 23.3 Hz), 146.36–143.72 (m), 137.48 (d, J = 1.3 Hz), 124.91 (d, J = 3.4 Hz), 123.57 (d, J = 7.1 Hz), 117.85 (d, J = 5.2 Hz), 107.40 (d, J = 8.5 Hz); ^{19}F NMR (400 MHz, DMSO) δ –165.19

5.3 Synthesis of Trifluoromethylated Flavonoids



3-(Trifluoromethyl)-2-(3,4,5-trihydroxyphenyl)-4H-chromen-4-one 15. A mixture of **2**-(3,4,5-trihydroxyphenyl)-4H-chromen-4-one **5** (10 mg, 0.032 mmol), copper(I) iodide (0.6 mg, 0.0032 mmol) and 5-(trifluoromethyl)dibenzothiophenium trifluoromethanesulfonate (Umemoto's reagent) (10 mg, 0.048 mmol) in DMF (1 mL) was heated at 80 °C under argon. After 34 h, the resultant mixture was extracted with diethyl ether (2 mL × 5). The organics were dried over Na_2SO_4 and concentrated under reduced pressure to afford the 3-(trifluoromethyl)-2-(3,4,5-trihydroxyphenyl)-4H-chromen-4-one. 20% estimated ^{19}F NMR yield; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.24 (d, *J* = 7.9 Hz, 1H), 7.72 (t, *J* = 7.7 Hz, 1H), 7.60 (d, *J* = 8.3 Hz, 1H), 7.44 (t, *J* = 7.3 Hz, 1H), 7.15 (s, 2H), 3.97 (s, 6H), 3.94 (s, 3H); ^{19}F NMR (300 MHz, $CDCl_3$) –56 (s, 3F).

5.4 The DPPH antioxidant assay

0.2 M stock solution of DPPH in 1:1 ethanol and acetone solution was prepared, and also, 1 mg/mL stock solution of the flavones in 1:1 ethanol and acetone solution. The positive control was vitamin C and it was prepared with concertation of 1 mg/mL in 1:1 ethanol and acetone solution. All the stock solutions were prepared daily and stored in the refrigerator in dark.

5.4.1 Preparation:

Fill all the wells with 100 μL of (1:1) ethanol-acetone solution then add to (A2 to A7) of the well 100 μL of the stock solution of the test flavones and prepare a serial dilution of (200, 100, 50, 25, 12.5, 6.25 and 3.125 μg/mL) by using the appropriate size of micropipette moving from A to H as seen in (Table 5.1). Next add 100 μL of DPPH solution to all the wells except the blanks, the plate must be covered all time to minimize evaporation and kept away from light in room temperature and left for 20 minutes then measure the absorbance at 517 nm

5.4.2 Calculation

% DPPH scavenging = [1-(Abs Sample with DPPH–Abs Sample Blank)]/[(Abs DPPH) – (Abs Solvent)] x 100

Table 5.1: 96-well plate arrangement

	1	2	3	4	5	6	7
	Control	Sample 1 (Blank)	Sample 1 + DPPH	Sample 1 (Blank)	Sample 1 + DPPH	Sample 1 (Blank)	Sample 1 + DPPH
A	DPPH 0.1 mM	Sample 1 200 µg/mL	Sample 1 200 µg/mL	Sample 1 200 µg/mL	Sample 1 200 µg/mL	Sample 1 200 µg/mL	Sample 1 200 µg/mL
B	DPPH 0.1 mM	Sample 1 100 µg/mL	Sample 1 100 µg/mL	Sample 1 100 µg/mL	Sample 1 100 µg/mL	Sample 1 100 µg/mL	Sample 1 100 µg/mL
C	DPPH 0.1 mM	Sample 1 50 µg/mL	Sample 1 50 µg/mL	Sample 1 50 µg/mL	Sample 1 50 µg/mL	Sample 1 50 µg/mL	Sample 1 50 µg/mL
D	DPPH 0.1 mM	Sample 1 25 µg/mL	Sample 1 25 µg/mL	Sample 1 25 µg/mL	Sample 1 25 µg/mL	Sample 1 25 µg/mL	Sample 1 25 µg/mL
E	DPPH 0.1 mM	Sample 1 12.5 µg/mL	Sample 1 12.5 µg/mL	Sample 1 12.5 µg/mL	Sample 1 12.5 µg/mL	Sample 1 12.5 µg/mL	Sample 1 12.5 µg/mL
F	DPPH 0.1 mM	Sample 1 6.25 µg/mL	Sample 1 6.25 µg/mL	Sample 1 6.25 µg/mL	Sample 1 6.25 µg/mL	Sample 1 6.25 µg/mL	Sample 1 6.25 µg/mL
H	DPPH 0.1 mM	Sample 1 3.125 µg/mL	Sample 1 3.125 µg/mL	Sample 1 3.125 µg/mL	Sample 1 3.125 µg/mL	Sample 1 3.125 µg/mL	Sample 1 3.125 µg/mL

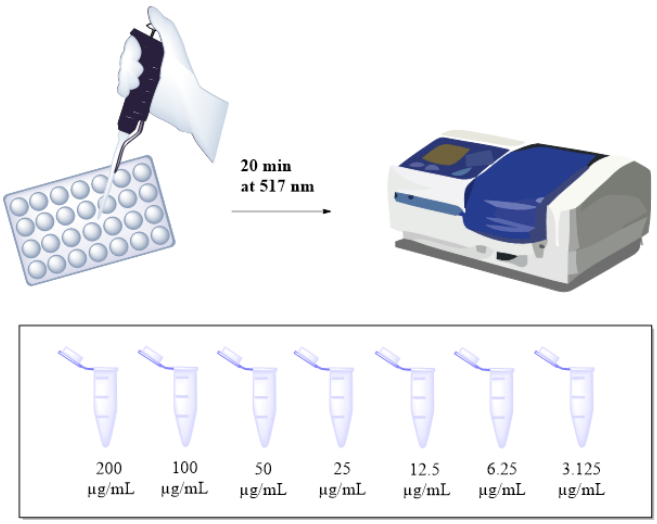


Figure 5.1: Procedure for DPPH assay

5.5 Neuroprotective assay

Before culturing the neurons, the 96-well plate was coated with 60 µL of 0.01% Poly-L-Lysine and incubated for 24 hours then washed with distilled water. Next, the neurons were cultured in a 96-well plate with 100 µL growth media that include neurobasal media, L-glutamine, fetal bovine serum (FBS), and Pen Strep and incubated for 24 hours. The growth media was replaced by a media that doesn't contain the fetal bovine serum (FBS), and the plate was then incubated for one week. Next, it was treated with glutamate and the test compound. After 24 h the MTS assay was performed by using CellTiter 96® AQueous One Solution Reagent.

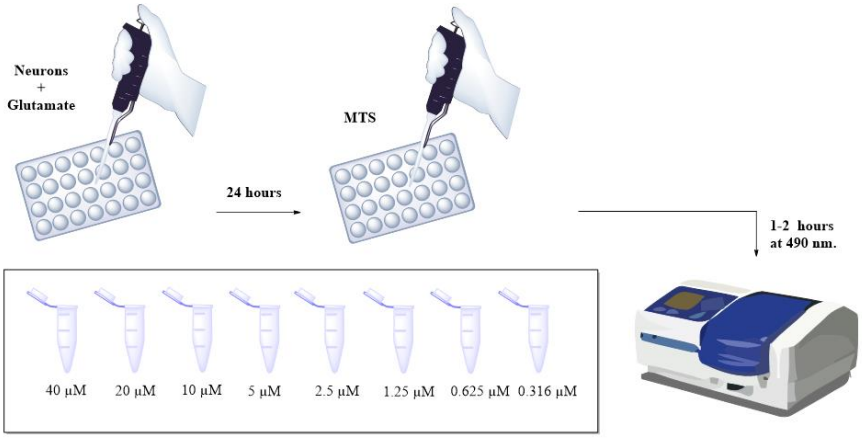


Figure 5.2: Procedure for neuroprotective assay

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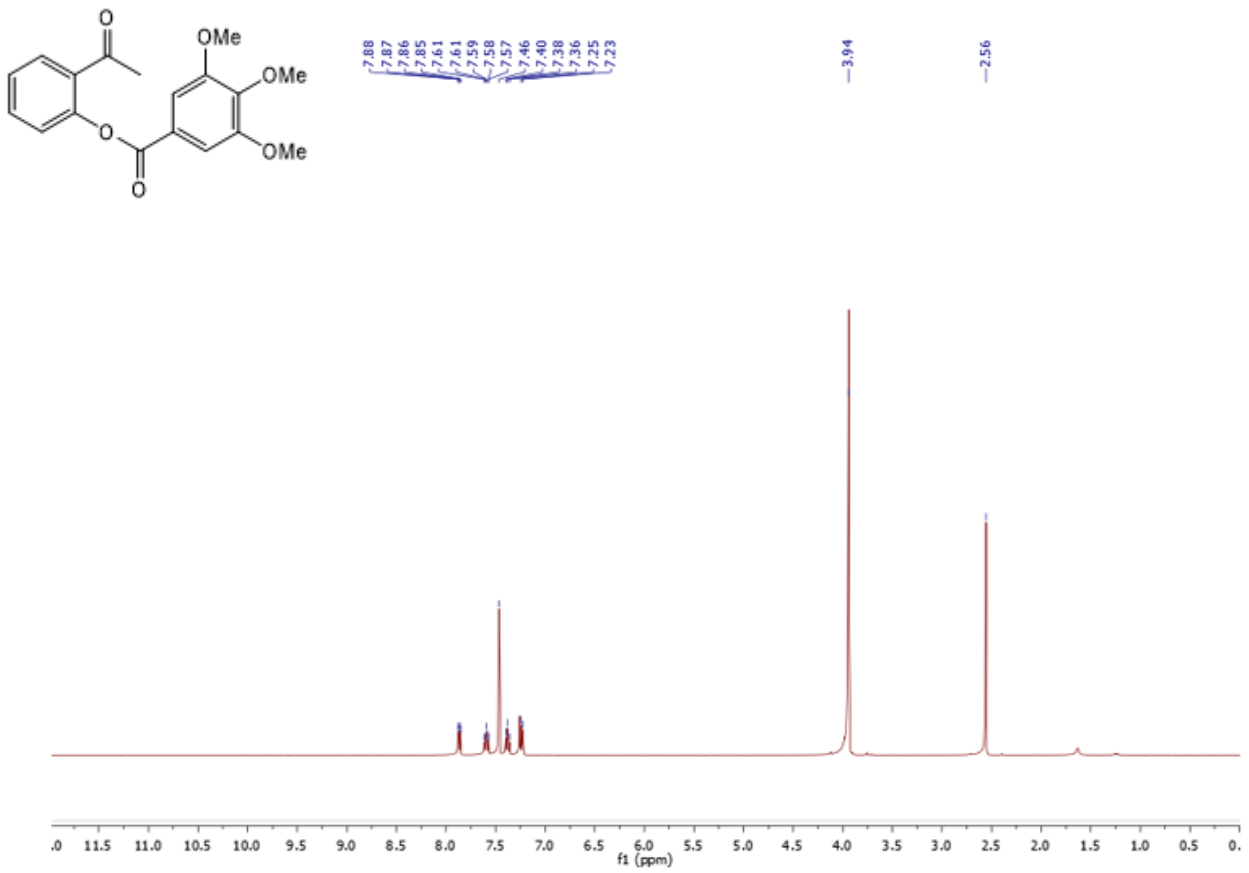
Appendix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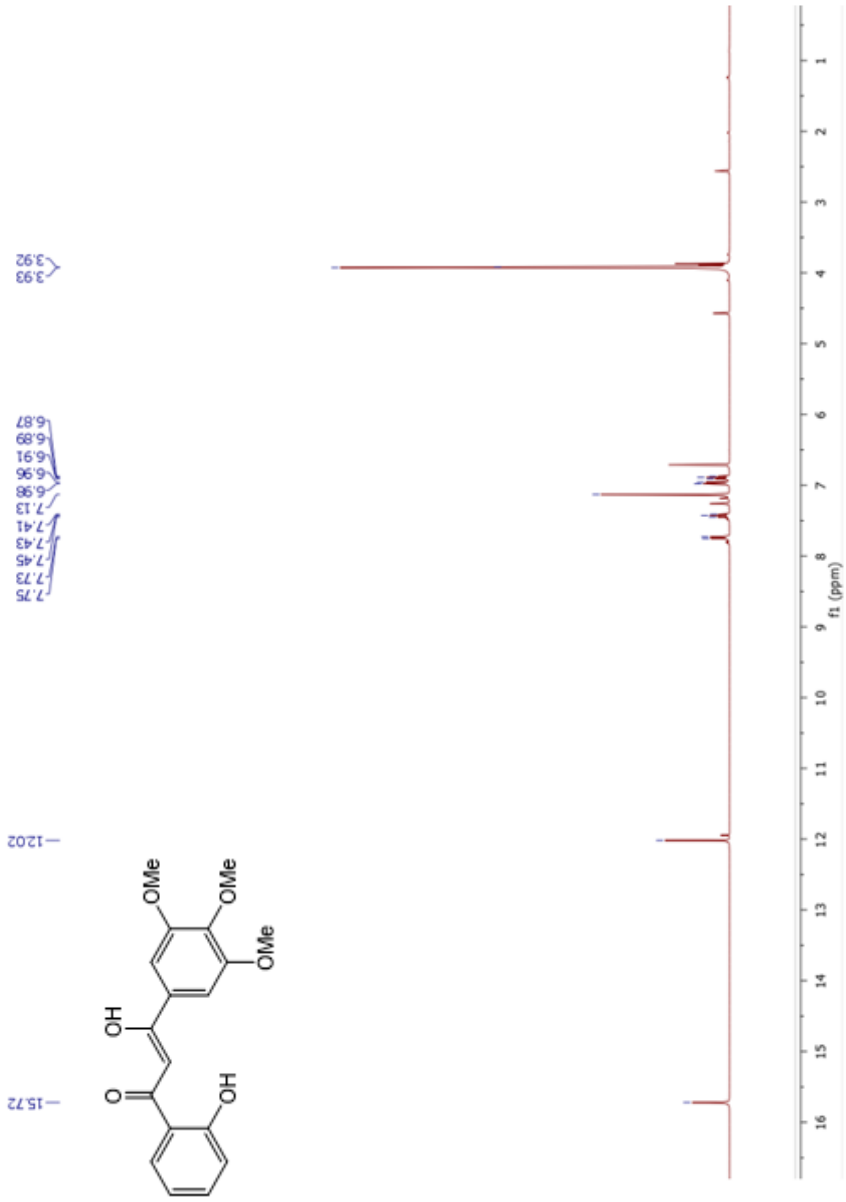
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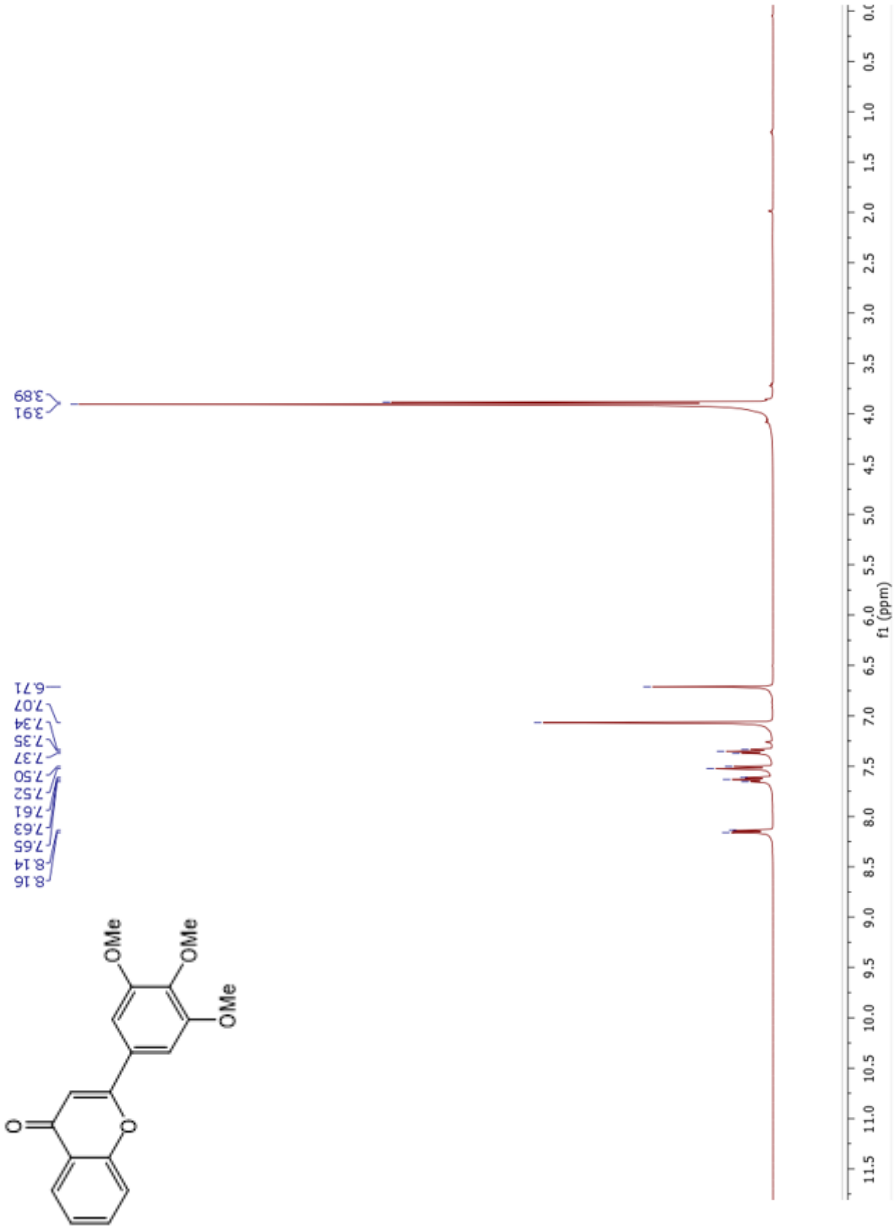
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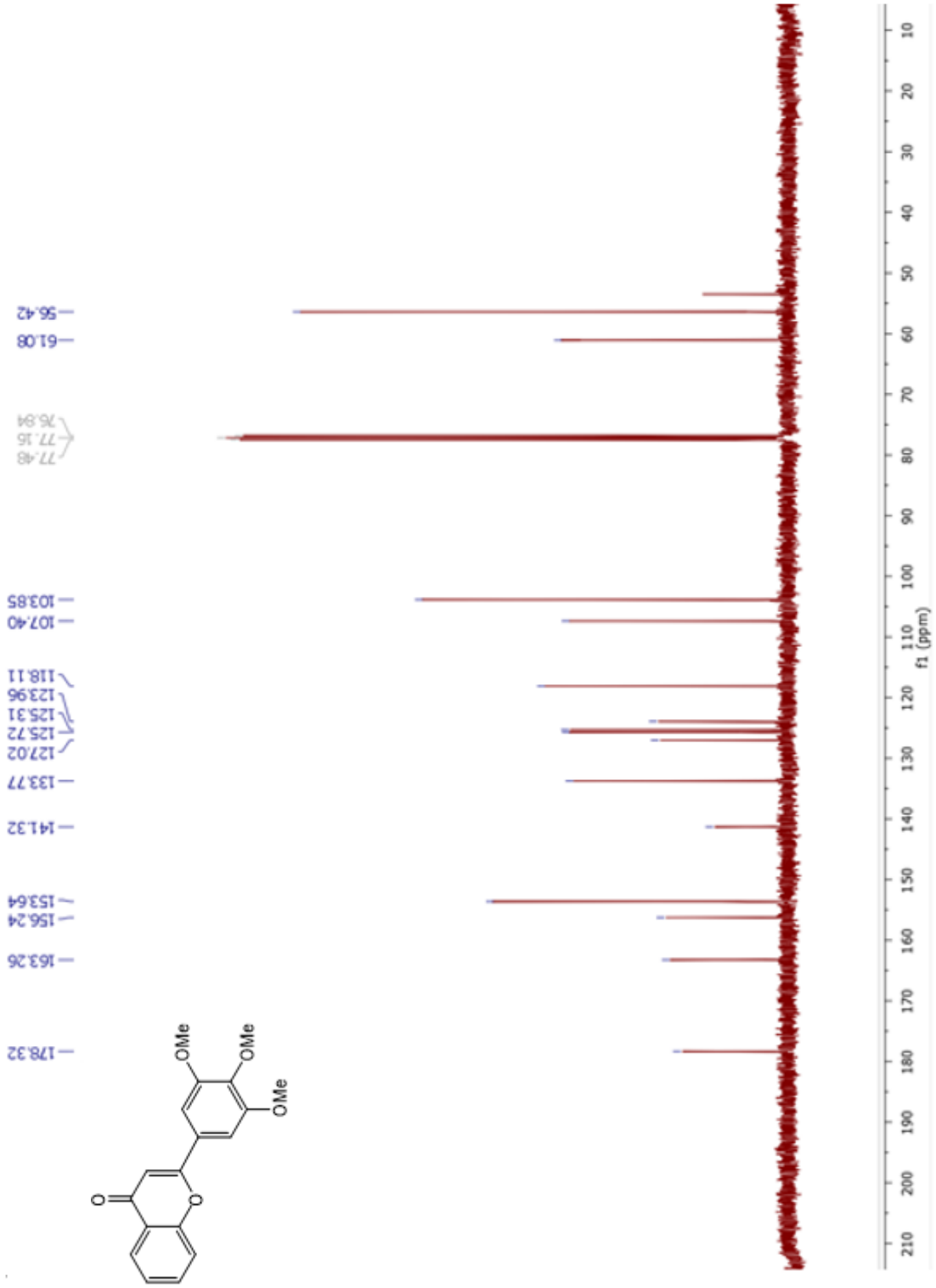
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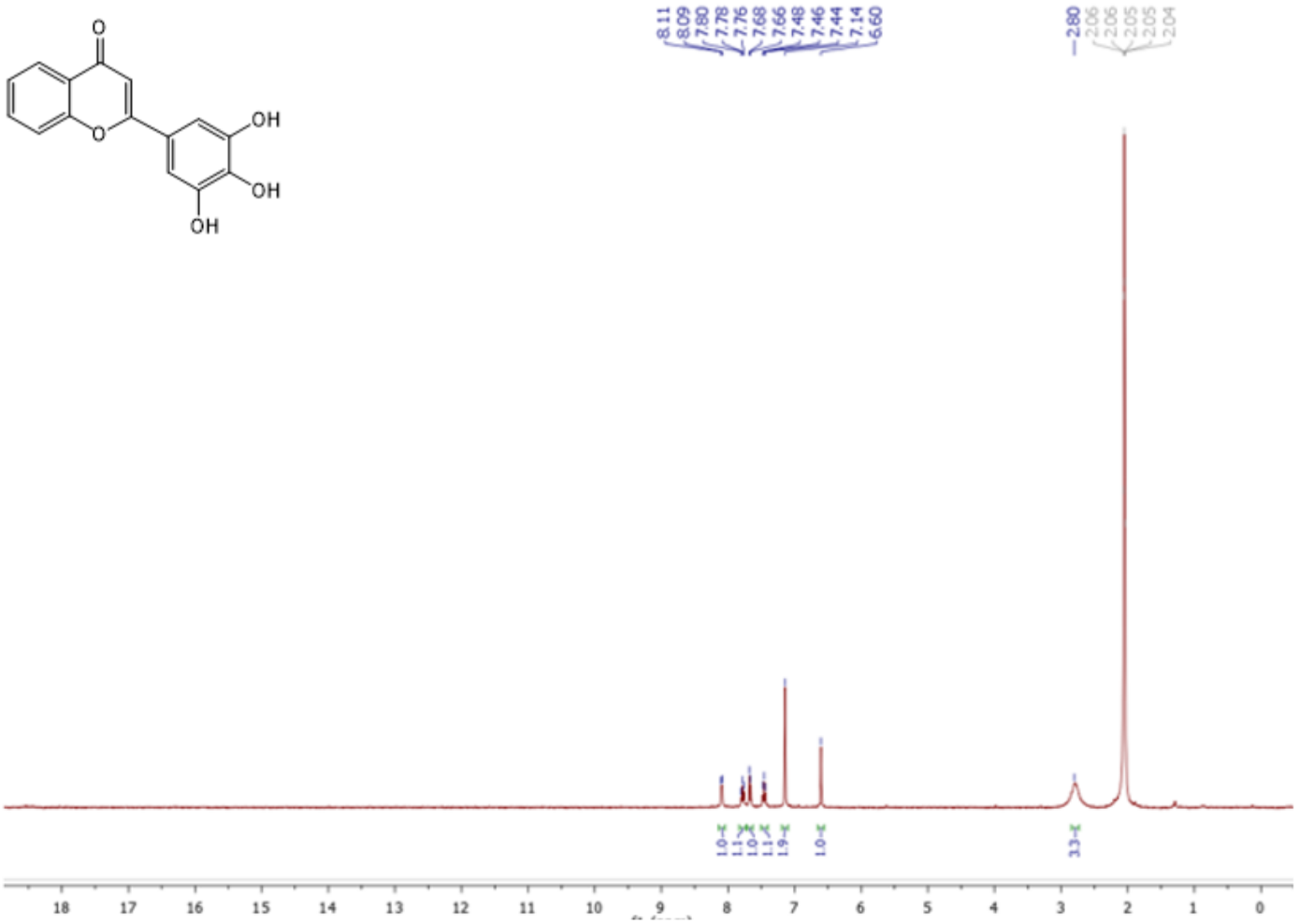
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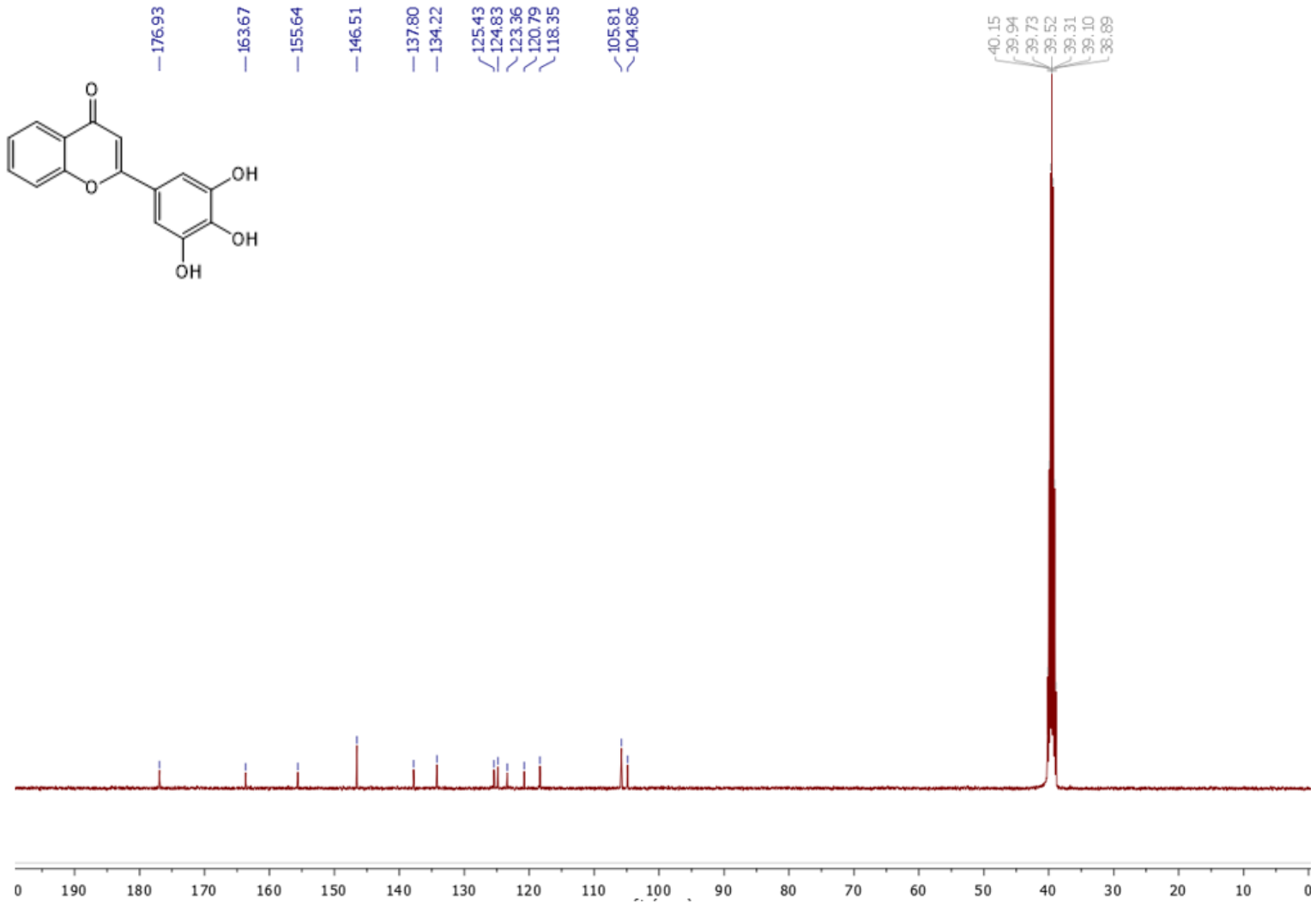
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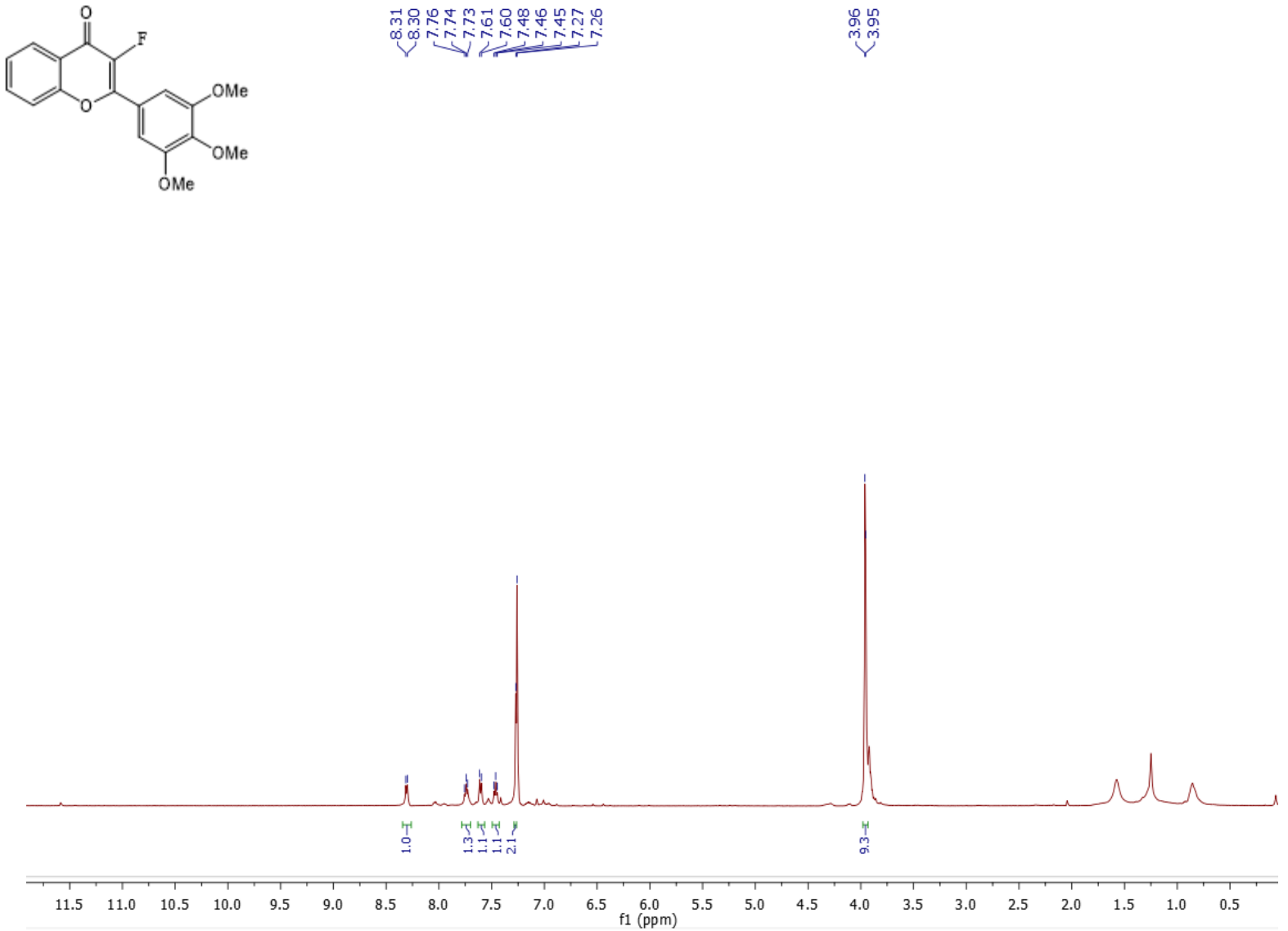
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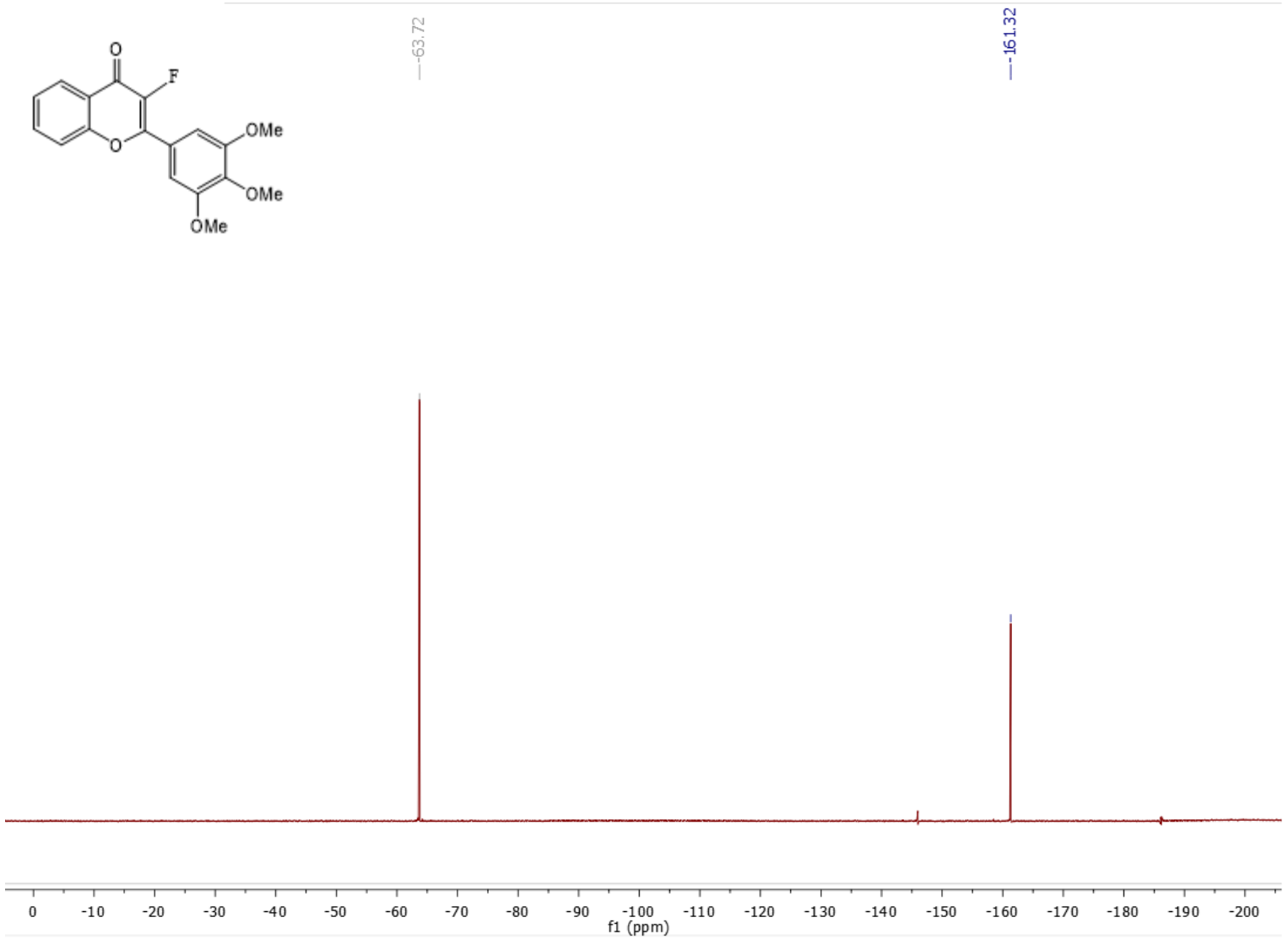
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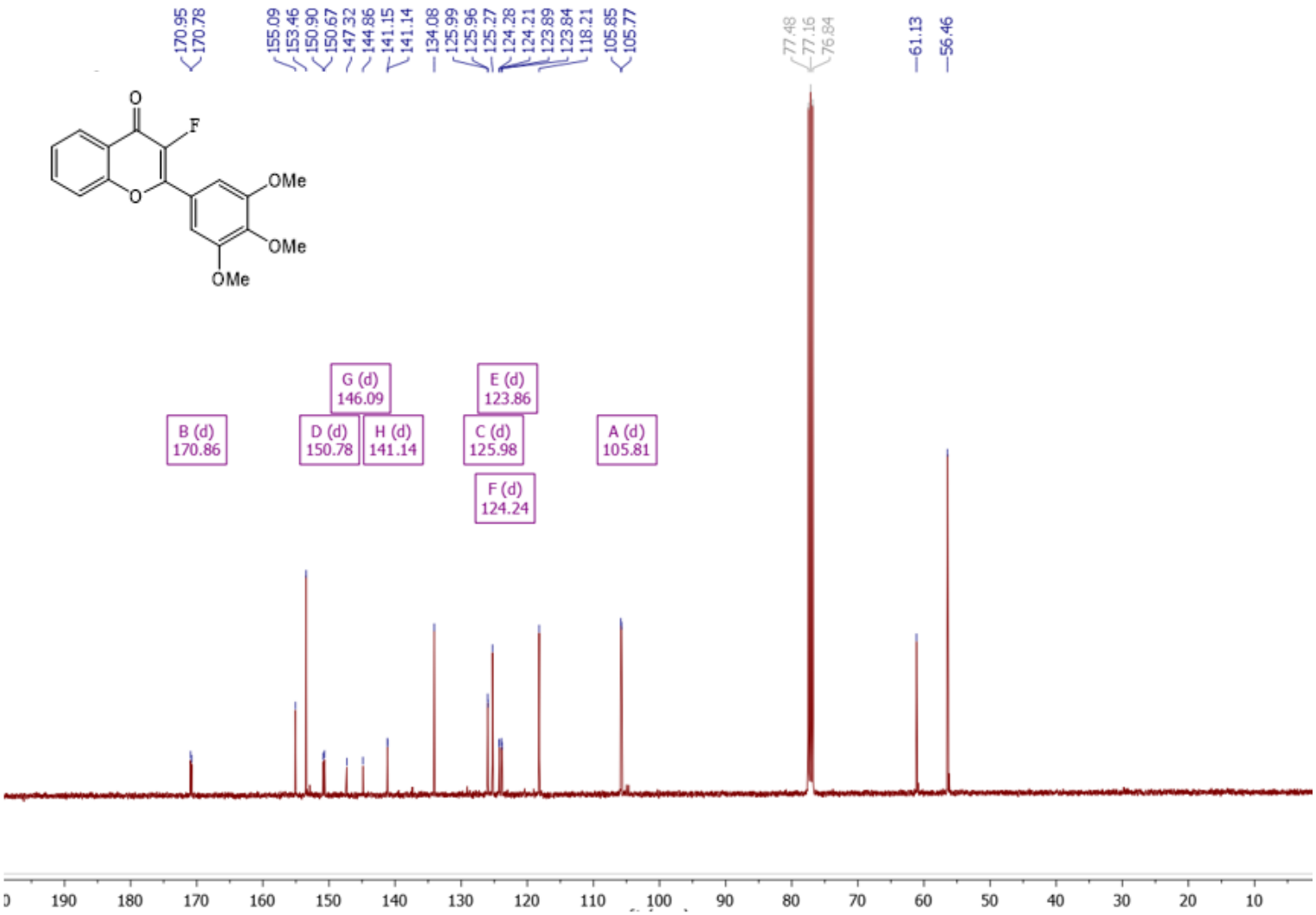
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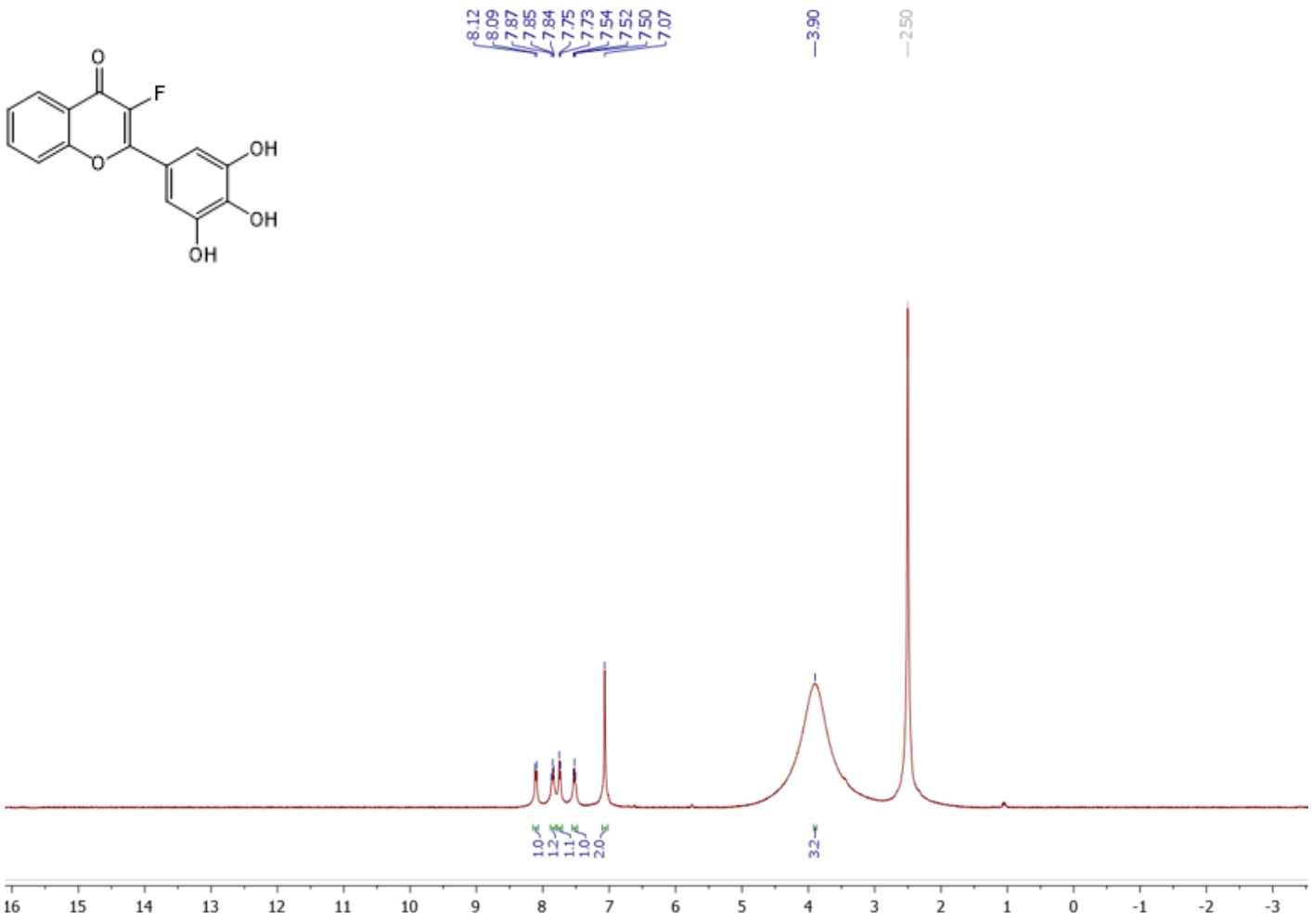
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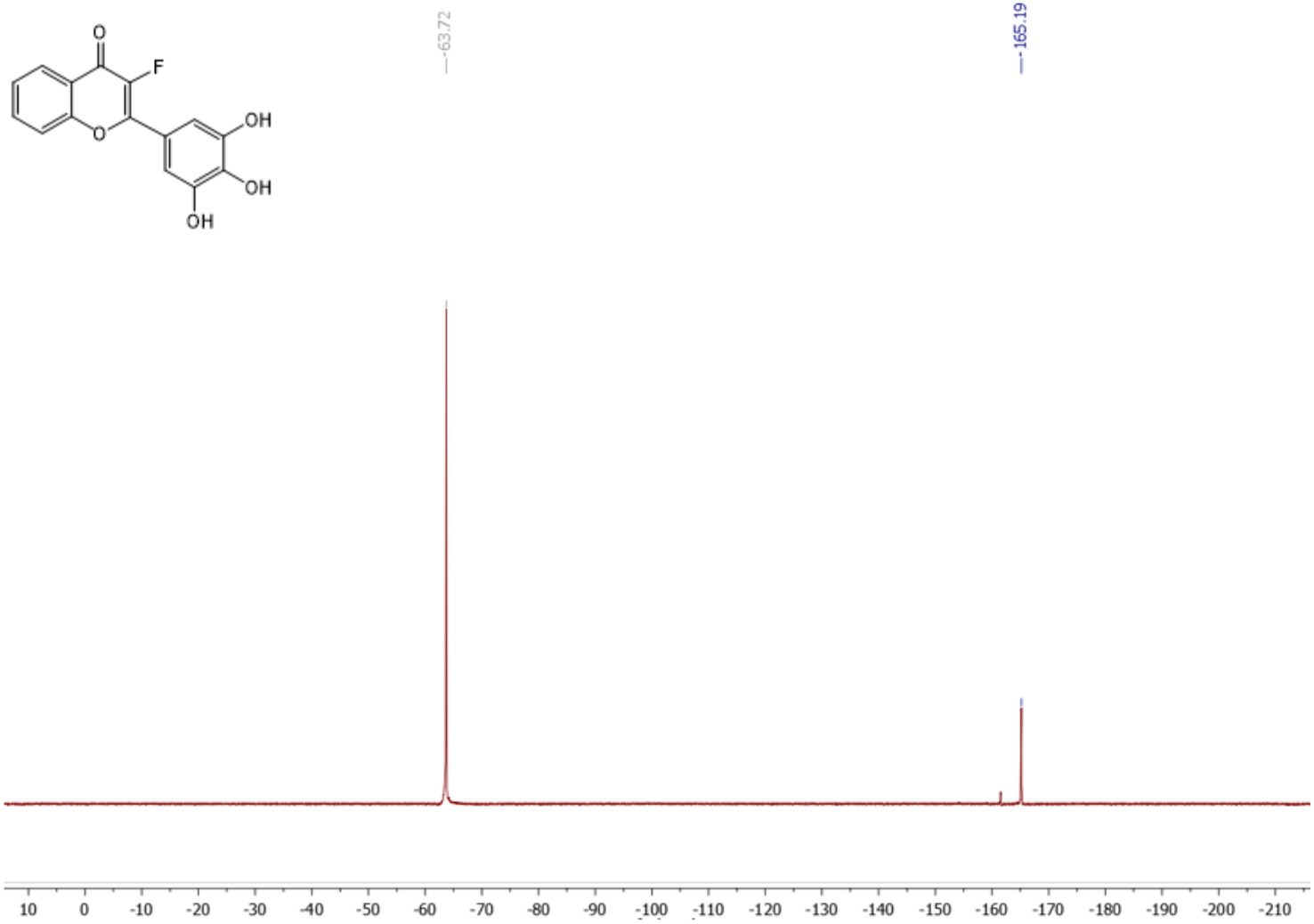
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VITA

Maali Alshammari was born in Riyadh, the capital of Saudi Arabia on June 27th, 1993. In 2016 she received her bachelor's degree in Pharmaceutical Sciences from the pharmacy school of King Saud university in Riyadh. In spring of 2017, Maali joined the lab of Professor David A. Colby in the Department of BioMolecular Sciences at University of Mississippi as a master's student in the medicinal chemistry program.